Conformation and Self-Diffusion of Single DNA Molecules Confined to Two Dimensions

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The conformation of DNA molecules electrostatically bound to fluid cationic lipid bilayers is investigated by fluorescence microscopy. The DNA diffuses freely in the plane and follows Rouse dynamics, $D \sim 1/N$, with an increasing number of base pairs N. The chain extension scales as $\langle R^2 \rangle \sim N^{2\nu}$, with $\nu = 0.79 \pm 0.04$ in good agreement with the exact exponent $\nu = 3/4$ for a self-avoiding random walk in two dimensions. The structure factor of dilute and semidilute DNA solutions shows fractal scaling behavior, $S(k) \sim k^{-1/\nu}$. In highly concentrated two-dimensional DNA solutions, the chains were found to segregate. [S0031-9007(99)08573-7]

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Polymers in two dimensions are theoretically appealing, but experimentally difficult to realize. Practical examples of polymers confined to two dimensions are thin polymer films, polymers adsorbed to surfaces, or polymers trapped in layered materials. Yet, most of these systems are not confined to an extent that they can be considered quasi-two-dimensional. On the other hand, static scaling laws for polymers in two dimensions with excluded volume interaction are theoretically well established [1]. Furthermore, extensive Monte Carlo simulations exist that provide a detailed analysis of the static and dynamic properties of 2D polymer chains [2,3]. In this Letter, we report on strongly adsorbed, but laterally free, DNA molecules, which provide comprehensive evidence for the predictions made in two-dimensional (2D) polymer statistics. The DNA monolayers studied here are confined to the surface of a fluid lipid membrane. The molecules exhibit an excluded volume effect that prevents any out-of-plane crossings. In particular, we see for the first time experimentally the collapse of the chain extension in concentrated solutions in two dimensions.

Fluorescently labeled DNA molecules are bound to fluid cationic lipid bilayers on glass substrates without inhibiting the lateral DNA mobility [Fig. 1(a)]. The chain conformations of equilibrated layers are imaged using fluorescence microscopy and analyzed by image processing. It has been shown previously that polymer behavior of fluorescently labeled biopolymers can be directly studied by means of real time optical microscopy [4]. During the last several years, phenomena such as reptation, entropic forces, and conformational changes have been unveiled on the single molecule level [5].

Substrate supported membranes were prepared on glass cover slips by vesicle fusion [6]. The glass slide was cleaned, incubated with sonicated DOPC/DOTAP liposomes (dioleoylphosphatidyl-choline/dioleoyl-trimethyl-ammonium propane 10:1), and rinsed. DNA of λ phage (Boehringer) was labeled with TOTO-1 (Molecular Probes, Eugene) at a dye-to-base pair ratio 1:5. Subsequently, a dilute solution of labeled λ phage was added and adsorbed onto the supported membrane in 10 mM HEPES (pH7) and 10 mM NaCl. The adsorbed molecules were imaged with a cooled high resolution 12 bit CCD camera (Princeton Instruments). Figure 1(b) shows a typical time sequence of a single λ-DNA molecule. The conformational changes of the molecule are slow compared to the frame rest of the camera due to the high viscosity of the oil-like membrane.

In the following, we assume that the fluorescence intensity represents the distribution of chain segments of the adsorbed polymer chain [4]. Note that not all details of the chain conformation are resolved and some

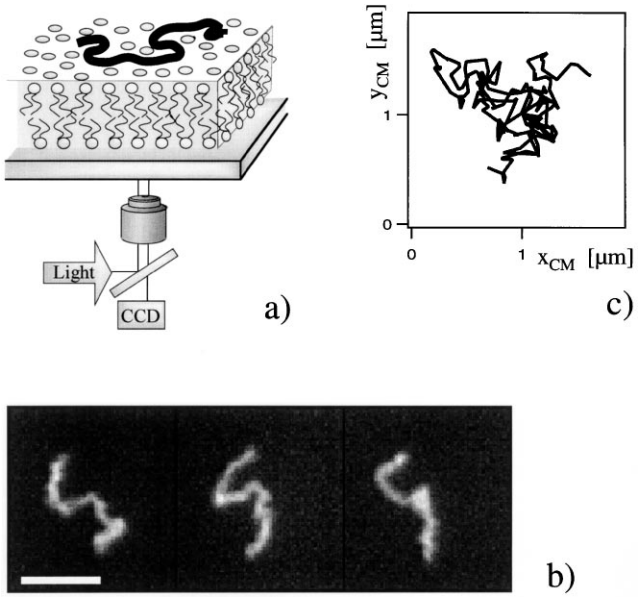

FIG. 1. Single λ-phage DNA molecule adsorbed onto a cationic lipid bilayer supported on a glass substrate. (a) Schematic (not to scale) sketch of the experimental setup. (b) Time series of fluorescence images at 2 sec intervals (bar represents 10 μm). (c) The center-of-mass motion of a 10.090 bp λ-DNA fragment following diffusive behavior.

folded parts with higher monomer concentration appear as brighter spots. However, this does not limit the analysis of single chains in terms of moments of the fluorescence intensity distribution, $I(x,t)$. The expansion yields the total fluorescence "mass" M_F, the center of mass $\mathbf{R}_{\mathrm{CM}}$, and the radius of gyration R_G,

$$M_F(t) = \int I(\mathbf{x},t)\, d\mathbf{x}\,,$$

$$\mathbf{R}_{\mathrm{CM}}(t) = \frac{1}{M_F(t)} \int \mathbf{x} I(\mathbf{x},t)\, d\mathbf{x}\,,$$

$$R_G^2(t) = \frac{1}{M_F(t)} \int |\mathbf{x} - \mathbf{R}_{\mathrm{CM}}|^2 I(\mathbf{x},t)\, d\mathbf{x}\,.$$

Each of these moments evolves in time, and corresponding time correlation functions can be determined for long enough time sequences. The first and second moments are normalized with respect to the total fluorescence intensity, which decays exponentially in the course of photobleaching. The center of mass follows a random walk as indicated in Fig. 1(c) and the self-diffusion constant is determined from the mean-square displacement vs time

$$\langle |\mathbf{R}_{\mathrm{CM}}(t) - \mathbf{R}_{\mathrm{CM}}(0)|^2 \rangle = 4Dt\,.$$

Fragments of λ DNA were prepared using restriction enzymes (ApaI, BbrPI, SacI, XbaI) in order to analyze the properties of a single flexible chain as a function of length. The log-log presentation of the lateral diffusion constant versus the number of base pairs N is shown in Fig. 2(a). The best linear fit of log D vs log N gives a slope -0.95 ± 0.06. This behavior is predicted by the Rouse model [7], which assumes that the friction, ζ, of the chain segments is localized and, hence, $D = kT/\zeta N$. The DNA is strongly coupled to the membrane and may be treated similar to a polymer in a two-dimensional fluid [8]. In the present case, the 2D fluid is free from Stokes paradox due to the hydrodynamic screening of the wall. A thin ($\delta_{\mathrm{H_2O}} \approx 10$ Å) lubricating water film between solid and membrane yields a hydrodynamic screening length $\xi = (\tilde{\eta}_M/\gamma_s)^{1/2}$ with slip coefficient $\gamma_s = \eta_{\mathrm{H_2O}}/\delta_{\mathrm{H_2O}}$ and $\tilde{\eta}_M$ being the 2D viscosity of the membrane. Assuming $\tilde{\eta}_M \approx 2 \times 10^{-10}$ N s/m, the screening length is $\xi \approx 140$ Å. The measured friction $\zeta = 8.3 \times 10^{-11}$ N s/m per base pair [from Fig. 2(a)] may be visualized as one lipid molecule being stuck to the DNA strand every helical turn, since effectively DNA pieces of 5-10 base pair length diffuse with the same self-diffusion constant as lipid.

The radius of gyration follows a power law $\langle R^2 \rangle \sim N^{2\nu}$ with the scaling exponent $\nu = 0.79 \pm 0.04$ as shown in Fig. 2(b). The number of base pairs N covers a range corresponding to 2.0–20 μm contour length of the DNA. The persistence length of DNA is known to be about 50 nm, meaning that λ-phage DNA [48.000 base pairs (bp)] consists of $\tilde{N} = 180$ statistically independent segments of length l_K (Kuhn segments). Hence, the static chain conformation of the adsorbed DNA might be expected to behave similar to a random coil with $\nu = \frac{1}{2}$. However, the chain extension and scaling exponent are larger due to repulsive monomer-monomer interaction. Consequently, the DNA is expected to scale with the known Flory-Edwards exponent $\nu = \frac{3}{4}$ for a self-avoiding chain in two dimensions. The later exponent is exact within self-consistent theories and renormalization-group arguments [1] and is indeed in agreement with the experiment. Note that also the experimental standard deviation of R_G [error bars in Fig. 2(b)] are of order of the expected static fluctuations. We find $\Delta R_G/R_G \approx 0.2$, comparable to the results seen in Monte Carlo studies [2]. Within Flory's lattice model $R_G = (a_F^{1/2} l_K/4)^{1/2} \tilde{N}^{3/4}$ we find an excluded area, $a_F = 3.8 \times 10^5$ Å^2, or equivalently an

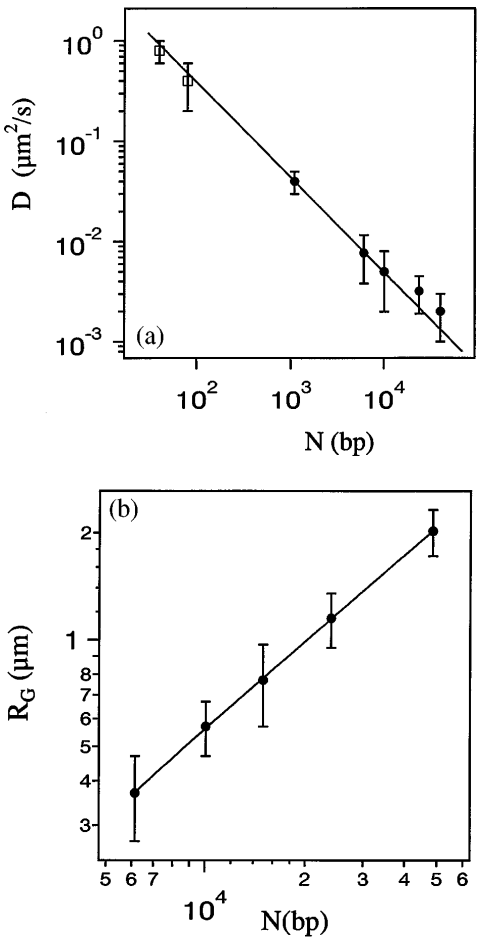

FIG. 2. Scaling behavior of λ-phage DNA fragments shown in log-log plots. (a) The self-diffusion coefficient versus number of base pairs. The diffusion constant was obtained from the center-of-mass time correlation function of single molecules, except for the two very short fragments (□), which were measured by fluorescence recovery of adsorbed monolayers of 40 and 80 bp double-stranded oligonucleotides [Zantl *et al.* (to be published)]. (b) The radius of gyration averaged over about 30 single chains as a function of the number of base pairs. The straight line exhibits slope 0.78.

effective width $\xi_\perp = a_F/l_k \approx 380$ Å of the DNA strand on the lipid bilayer. The excluded area may be derived as a virial coefficient of interacting charged rods. However, the electrostatic repulsion between segments of bound nucleic acid is more elaborate and turns out to be screened by cationic lipids playing the role of counterions [9].

A larger surface coverage of adsorbed DNA molecules may be considered a 2D solution of interacting polymers in an effective 2D lipid solvent. Ensembles of different densities, corresponding to dilute, semidilute, or concentrated 2D-DNA solutions, are prepared by incubating DNA solutions of variable concentrations for a short period of time. The samples equilibrate after 24–48 h and exhibit homogeneous number densities of molecules [Fig. 3(a)]. The structural properties of the 2D solutions are well described in Fourier space, as acquainted from scattering techniques. Here, the so-called static structure factor is calculated by image processing:

$$S(k) = \left\langle \left| \sum I(\mathbf{x}) e^{i\mathbf{k}\cdot\mathbf{x}} \right|^2 \right\rangle_\varphi ,$$

where k denotes the absolute value of the scattering vector $\mathbf{k}$, and φ denotes the circular average in plane. The structure factor is furthermore averaged over several images to improve the statistics and is divided by the optical transfer function OTF(k) to correct for the finite optical resolution. The later was determined in an independent calibration experiment using submicron latex beads. As shown in Fig. 3(b), the dilute and semidilute solutions exhibit fractal scaling behavior [1,2], $S(k) \propto k^{-1/\nu}$, with the scaling exponent $\nu = \frac{3}{4}$ in the regime, where we see the inside of the polymer coil, $1/R_G \leq k \leq k_{\max}$. Note that the scaling regime of the semidilute data is smaller than for the dilute case due to intermolecular correlations on length scales larger than the density screening length, which here is of order R_G. A Zimm plot, $S^{-1}(k) \propto 1 + k^2 R_G^2/2$ for small angle scattering of dilute solutions [see inset of Fig. 3(b)] yields the radius of gyration. The value $R_G = 1.7 \pm 0.2$ found for λ phage is close to the value measured from the direct second moment analysis of single molecules. Semidilute and concentrated structure factors show an unexpected increase of $S(k)$ in the small angle regime that is indicative of long-range correlations. These fluctuations cannot be explained at the moment, but seem to be dynamic and to vary over a long time. At the same time, the underlying lipid bilayer remains optically homogeneous as probed by fluorescently labeled lipids.

The chain conformation of DNA in a concentrated 2D-DNA solution is investigated by fully saturating the cationic surface with λ-phage DNA. In order to distinguish individual chains, only one in a hundred DNA molecules is fluorescently labeled. As demonstrated in Fig. 4(a), the chains are collapsed to dense spots with radius of gyration $R_G^{\text{conc}} = 0.7 \pm 0.2$ μm. No long-range diffusion is observed, but chain motion on the length scale smaller than R_G^{conc} is visible under the microscope.

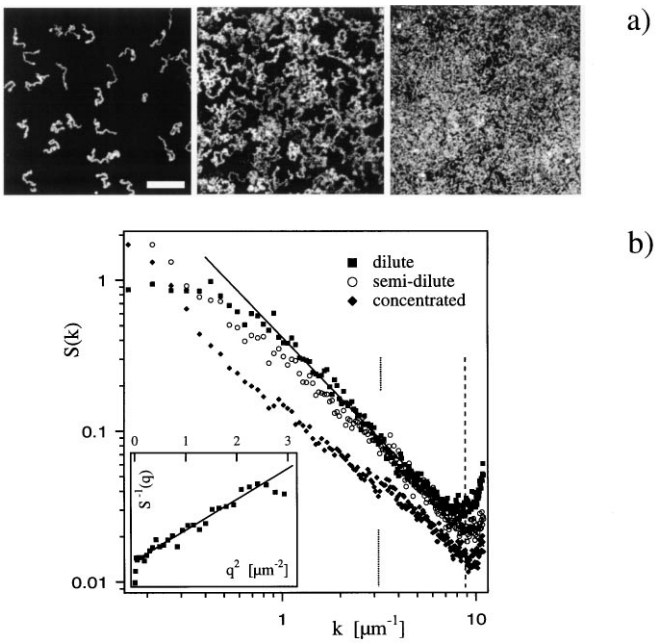

FIG. 3. (a) Real space micrographs of λ-phage DNA at three different concentrations (bar = 10 μm). (b) The corresponding structure factor of the dilute (■), semidilute (○), and concentrated (◆) regimes. The structure factors are determined by Fourier transformation over several fluorescence images in each regime. The Fourier-transformed images are circular averaged and divided by the empirically determined optical transfer function of the microscope. The full line follows slope $-4/3$ and the vertical dashed lines indicate the value of $1/R_G$ and the limit of optical resolution. The inset shows a Zimm plot, $S^{-1}(k)$ vs k^2, for the dilute data.

The collapse of R_G is in striking contrast to the behavior in concentrated solutions in three dimensions, where the chains adopt a random coil conformation due to screening. However, in two dimensions a large energetic penalty for crossings rules out entanglement and leads to segregation of chains [10]. The radius of gyration of a completely segregated chain, filling a disklike area with only its own base pairs, is $R_G^{\text{disk}} = \sqrt{N/2\pi c}$, with c being the concentration of base pairs. The base-pair concentration is fixed by the mol fraction of cationic lipid in the bilayer due to the requirement of local charge neutrality [11]. Hence, for a DNA chain on a membrane with 10% cationic lipid $c = 7 \times 10^4$ μm^{-2} and $R_G^{\text{disk}} \approx 0.3$. Since $R_G^{\text{conc}} > R_G^{\text{disk}}$, the chains in the concentrated regime are not completely segregated but slightly swollen. We demonstrate that the observed effect is not due to the increased concentration, but rather due to the topological mutual hindrance of the chains in two dimensions. The latter is lifted, if the majority of unlabeled DNA is chopped into short strands. Figure 4(b) shows the radius of gyration, $R_G^{\text{sol}} = 2.0 \pm 0.2$ for λ phage in the presence of unlabeled 40 bp double stranded oligonucleotides.

We showed that the static and dynamic properties of long DNA molecules adsorbed to cationic membranes

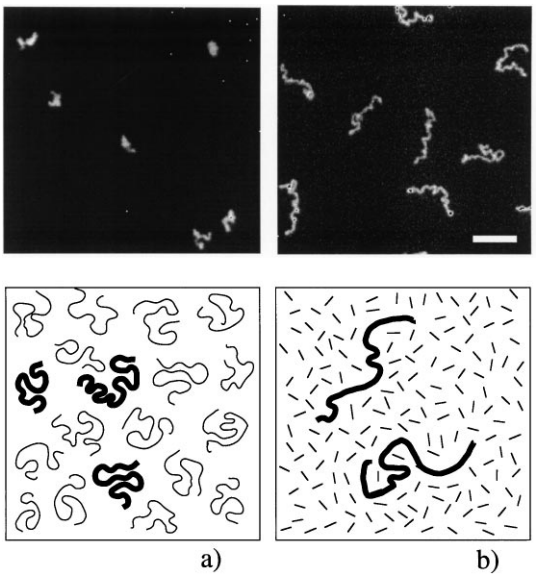

FIG. 4. Cationic bilayer fully saturated with concentrated two-dimensional DNA monolayer. (a) Only one in a hundred λ-phage DNA molecules is fluorescently labeled. The segregation of chains is visible by a reduced radius of gyration. (b) In contrast, λ-phage DNA molecule coadsorbed with an excess of 40 bp double stranded oligonucleotides (unlabeled) exhibit an extended chain conformation (bar = 10 μm).

can be studied by optical microscopy almost down to the length of one Kuhn segment. However, on smaller length scales, accessible by scanning force microscopy, the conformations of a single DNA molecule were shown to be appropriately described by the wormlike chain model [12]. Furthermore, the present study was limited to the case of low cationic lipid mol percentage. On highly cationic membranes the concentrated 2D-DNA phase is even more densely packed and eventually exhibits local smectic ordering [11,13]. In quintessence, dilute ensembles of DNA bound to cationic membranes are perfectly described by self-avoiding walks on the optical length scale. They provide a model system for two-dimensional polymer solutions, which can be used in the future to explore also conformational relaxation times, density correlation functions, and phase behavior in two dimensions. For this purpose, cationic membranes provide a unique, defect-free surface with control over frictional coupling and lateral interactions.

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