

Stretching DNA with a Receding Meniscus: Experiments and Models

D. Bensimon,¹ A. J. Simon,^{1,2} V. Croquette,¹ and A. Bensimon³

¹Laboratoire de Physique Statistique, Ecole Normale Supérieure, 24 rue Lhomond, 75231 Paris Cedex 05, France

²Laboratoire de Physique de la Matière Condensée, Ecole Normale Supérieure, 24 rue Lhomond, 75231 Paris Cedex 05, France

³Laboratoire de Biophysique de l'ADN, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France

(Received 13 September 1994)

A detailed experimental and theoretical analysis of the alignment of grafted DNA molecules by a moving meniscus is presented. The existence and extent of the stretching (up to 2.14 times the unstretched length) depends critically on the properties of the surface. Molecules grafted at both ends exhibit a looplike shape which is scale invariant. An elastic model of this process, which we have called molecular combing, is introduced which (a) yields the extension force on various surfaces, (b) yields a value for the tensile strength of DNA, 476 ± 84 pN, and (c) describes the shape of the loops with no fitting parameters.

PACS numbers: 87.15.-v, 82.65.-i, 87.90.+y

The study of polymer physics at the single molecule level is being greatly advanced by the visualization [1] and manipulation of single DNA molecules. Electrophoresis of single fluorescent DNA molecules has been studied in gels [2] and in microlithographic arrays [3]. Measurements on single DNA molecules [4] have been shown to fit remarkably well the elastic theory of stiff chain polymers [4]. Finally, single fluorescent molecules grafted to beads and manipulated by optical tweezers have been used to study the reptation and relaxation of polymers [5]. From a more applied point of view, various approaches have been tried to align DNA as a preparative step in the sequencing or mapping of a single molecule. Alignment of free molecules in a gel [6] or of grafted ones in a flow [7] have been proposed. In the same vein we have discovered a new phenomenon involving the systematic and complete alignment of grafted DNA molecules by the action of a receding meniscus. This phenomenon, called molecular combing, has been previously described and discussed from a more biological point of view [8]. Here we address the physical mechanism responsible for the extension of the molecule.

DNA molecules were grafted, as previously described, on a variety of surfaces: surfaces silanated with an exposed vinyl group (silanated surfaces) and used as such, or further coated with proteins [8], bovine serum albumin (BSA), protein A, or protein A/antidigoxigenine (antiDIG surfaces [9]). A unique feature of these techniques is that they ensure the grafting of the molecule at one or both extremities only, avoiding binding of the molecule along its length. DNA was stained with YOYO1 [dimer of oxazole yellow $(\text{CH}_2)_3\text{-N}(\text{CH}_3)_2\text{-(CH}_2)_3\text{-N}(\text{CH}_3)_2\text{-(CH}_2)_3$] [9] and observed, in solution or after passage of the meniscus, by video enhanced fluorescence microscopy.

A preliminary task was to assess whether the degree of extension of the molecule was due to its interaction with the dye or to the action of the meniscus. For that purpose, λ -DNA was incubated at various ratios of dye

molecules/base pairs (1:5, 1:10, and 1:20), grafted on identical silanated surfaces and combed. The probability distribution of the length (PDL) of the combed molecules is measured for each YOYO1/bp ratio. All PDL's exhibit the *same* peak at the largest extension, $\sim 24 \mu\text{m}$, as shown in Fig. 1(a) (for a ratio 1:5). This suggests that the extension of the molecule is mainly due to the

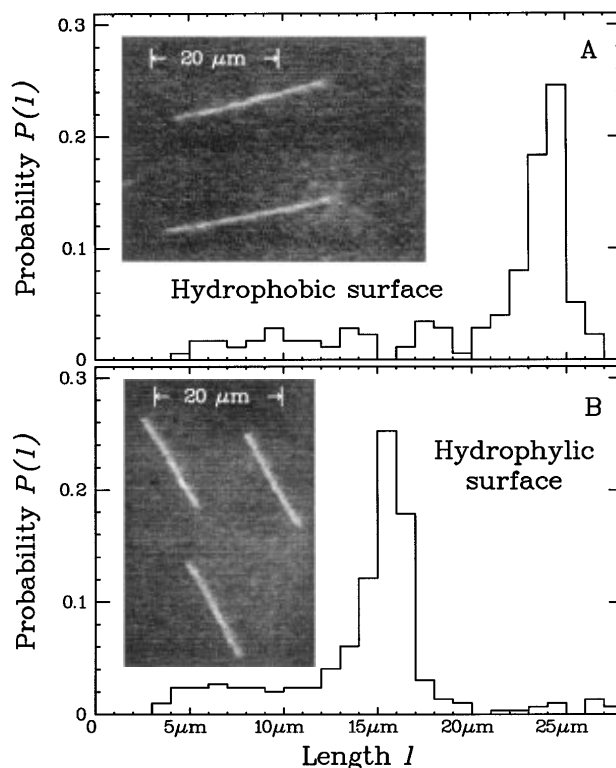


FIG. 1. Histograms of the length of N segments of λ -DNA segments and a typical image (inset) combed on (a) silanated surfaces in biological buffer 2-[N-morpholino] ethanesulfonic acid (MESS) 50 mM at $p\text{H} = 5.5$ ($N = 175$); (b) antiDIG surfaces in biological buffer (tris) [hydroxymethyl] aminoethane (TRIS) 10 mM at $p\text{H} = 7.5$ ($N = 297$).

tension of the receding meniscus which is strong enough to lengthen the DNA by about 50% (the unstretched extension of λ -DNA is $16.1 \mu\text{m}$). Random ligation of λ -DNA with DNA-ligase leading to the formation of multimers, followed by combing on silanated surfaces, yields equally spaced peaks in the PDL at the monomer, dimer, and trimer locations. Thus the degree of extension does not depend on the length of the molecule, implying that the tension acts locally in the vicinity of the meniscus.

The degree of extension, however, depends strongly on the surface treatment. The peak of the PDL for λ -DNA combed on different batches of silanated surfaces (nonwetting) was observed to vary between 21 and $24 \mu\text{m}$. Thus even small variations in the surface coating affect the degree of extension. Furthermore the peak of the PDL of DIG labeled [9] λ -DNA combed on different batches of antiDIG surfaces (wetting) was observed to vary between 16 and $18 \mu\text{m}$, see Fig. 1(b).

The combing is suppressed in the presence of strong nonspecific adsorption. Thus adsorption of DNA to antiDIG surface is observed by varying the surface properties: changing the pH or the coverage of protein. In solution the molecules bind to the surface, stop fluctuating, and cannot be combed. A similar behavior is observed on silanated surfaces coated with the protein BSA [10]. At high concentration of BSA ($100 \mu\text{g/ml}$) there is strong nonspecific adsorption of DNA. Then as the concentration is decreased, the molecules are first (at $10 \mu\text{g/ml}$) observed to be combed as on antiDIG surfaces (weak extension force), and then (at $1 \mu\text{g/ml}$) one recovers the behavior on silanated surfaces (strong extension force).

The force exerted on the molecule in solution thus results from a local competition between (1) the DNA/surface interactions in front of the meniscus (nonspecific adsorption) and (2) the local action of the meniscus on the molecule. The force can be reduced by the addition of surfactants (e.g., Tween 20). Notice that the stronger the nonspecific adsorption the less the molecule is stretched. Since the receding meniscus constrains the molecule in solution to the surface, it makes sense that the exerted tension will be stronger the smaller the affinity between the surface and the DNA. However, the molecule left dry behind the meniscus adheres strongly to the surface [11]. A molecular understanding of these interactions is a complex problem. In the following we shall *assume* that the end result of these interactions is a *constant stretching force parallel* to the direction of combing, exerted on the molecule in the vicinity of the contact line.

One may use the experimental observations and Hooke's law to estimate this force [12]. Let $2l$ and $2l_0$ be the stretched and unstretched length of the portion of the molecule in the vicinity of the meniscus. The force acting on the molecule is $F_{\perp} = EA(l/l_0 - 1)$, where $E = 1.1 \times 10^8 \text{ N/m}^2$ is its Young modulus [13] and $A = 3.8 \times 10^{-18} \text{ m}^2$ its cross sectional area. The relative extension l/l_0 , the local strain, can be deduced from the

ratio between the peak of the PDL and the natural length of the molecule ($16.1 \mu\text{m}$). On silanated surfaces one measures $l/l_0 = 1.38 \pm 0.11$ and thus $F_{\perp} \sim 160 \text{ pN}$, whereas on antiDIG surfaces $l/l_0 = 1.13 \pm 0.03$ and thus $F_{\perp} \sim 54 \text{ pN}$. These forces are stronger than typical entropic [4] or hydrodynamic [14] ones (a few pN) but are comparable to the bond strength between biotin and streptavidin [15].

DNA molecules grafted at both ends to a surface can also be combed. First as the meniscus moves past the anchoring points of the molecule it stretches its two anchored segments (legs) *perpendicular* to the interface, as described above. The portion of the molecule in solution decreases until it just spans the distance between the two legs. It is now stretched *parallel* to the contact line, its length diminishing as the meniscus recedes. The final shape adopted by the molecule is a loop connecting two straight segments, see Fig. 2. Although the size

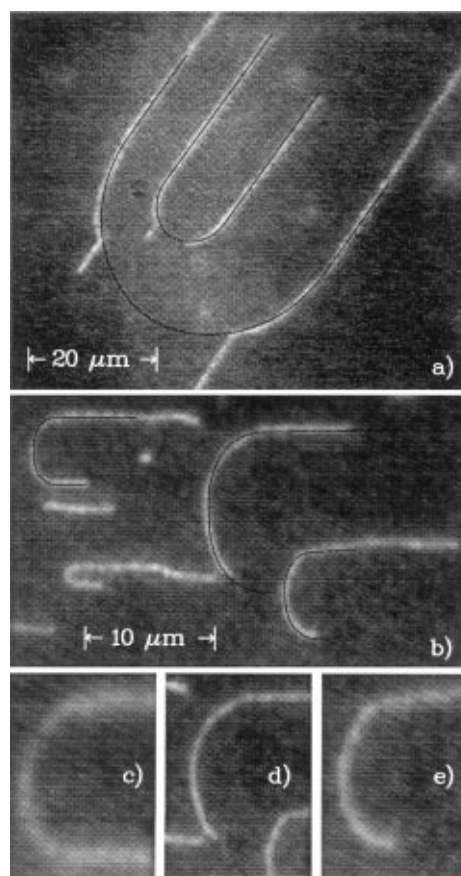


FIG. 2. Comparison between the observed and calculated shape of the loops. (a) Image of broken loops of fragments of *E. coli* DNA on silanated surfaces ($\text{pH} = 5.5$) with their theoretical shape superimposed ($a = 1.36$). The parameter a has been determined independently at the breaking point and via the peaks in the PDL (see text). (b) Image of unbroken loops of λ -DNA on antiDIG surfaces (at $\text{pH} = 7.5$) with their theoretical shape superimposed ($a = 0.47$). The scale is set by the perpendicular distance between the legs. (c), (d), and (e) correspond to three loops as in (b) but rescaled, demonstrating the scale invariance property.

of loops combed on a given surface can vary by a factor of 10 or more, their shape is identical: they are *scale invariant*, i.e., all loops can be superposed after rescaling by the perpendicular distance between their legs [Figs. 2(c), 2(d), and 2(e)]. If the tension is strong enough the loop breaks. This is always the case on silanated surfaces [see Fig. 2(a)] but never on antiDIG coated surfaces [Fig. 2(b)].

At the breaking point, we can measure the stretched and unstretched lengths of the molecule, l_b and $l_{0,b}$, respectively (see Fig. 3). An average over 26 broken loops yields $l_b/l_{0,b} = 2.14 \pm 0.20$. This elongation is very large; however, stretching of DNA to twice its length by hydrodynamic drag has been reported [16] and a 50% increase in length is known to be induced by Rec-A proteins binding to DNA [17]. Assuming that most of the stretching is done in the elastic regime (a pretty good assumption as we shall see below), one can infer from that measurement the tensile strength of DNA (stained with YOYO1), i.e., the force [18] F_b necessary to break it:

$$F_b = EA \left(\frac{l_b}{l_{0,b}} - 1 \right) = 476 \pm 84 \text{ pN}. \quad (1)$$

This measurement is compatible with the lower bound for the tensile strength of DNA determined by Harrington and Zimm [19]: 270 pN.

We shall now use the elastic framework introduced previously to describe the shape of the loops. Our model relies on the following assumptions: (1) The end result of the interactions within the meniscus region is a *constant* force F_0 acting along the *local* direction of the combed molecule [20]; (2) the component of F_0 parallel to the meniscus is balanced by the elastic force of the molecule, while its perpendicular component is balanced by the force exerted by the interface on the molecule; and (3) as soon as a part of the stretched molecule is laid in the dry region it sticks to the substrate.

From assumptions (1) and (2) we then have [see Fig. 4(a)]

$$F_x = F_0 \sin\theta(s) = EA \left[\frac{l(s)}{l_0(s)} - 1 \right], \quad (2)$$

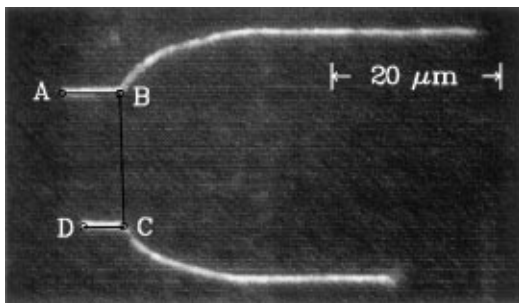


FIG. 3. Broken loop observed on silanated surfaces. From measurements on such figures we deduce the extension of DNA at the breaking point: the stretched molecule length is $l_b = \overline{BC}$, its unstretched length $l_{0,b}$ is deduced by dividing the length $\overline{AB} + \overline{DC}$ by the extension factor observed for straight molecule combed on the same surface.

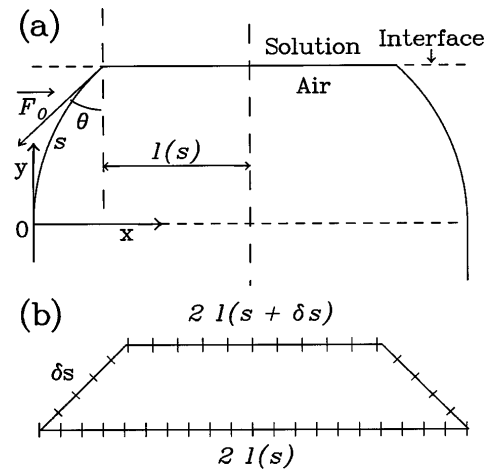


FIG. 4. Schematic drawing of a loop in formation. (a) F_0 is the constant tangential force exerted on the molecule, $2l(s)$ is the length of the portion of molecule still in solution, and s the arclength from the origin O. (b) Schematic drawing exhibiting the relation between $l(s)$ and $l_0(s)$ (the unstretched length of the molecule still in solution which is proportional to the number of stretched bp's symbolized by tick marks and their values after the molecule has been combed by an amount δs). The amount of bp's in δs is proportional to $l_0(s)\delta s/l(s)$.

where s , the arclength or intrinsic coordinate along the combed portion of the loop, serves as a convenient parametrization. Initially we normalize our lengths so that $l(0) = l_0(0) = 1$. As the meniscus recedes a growing portion of the molecule is combed and both $l(s)$ and $l_0(s)$ decrease. From assumption (3) one can see [Fig. 4(b)] that the portion of unstretched length $l_0(s)$ (i.e., the number of bp's) left combed by the receding meniscus obeys

$$l_0(s) = l_0(s + \delta s) + \frac{\delta s}{l(s)} l_0(s). \quad (3)$$

Setting $\sin\theta = -dl/ds$, one obtains the following set of differential equations:

$$\frac{F_0}{EA} \frac{dl}{ds} = 1 - \frac{l(s)}{l_0(s)}, \quad \frac{dl_0}{ds} = -\frac{l_0(s)}{l(s)}. \quad (4)$$

These equations are scale invariant, accounting for the self-similarity of the loops. Other models we have tried have been unable to account for the scale invariance property of the loops (if F_0 is proportional to l) or their smooth tip (if F_y is constant). Introducing $a \equiv F_0/EA$, the solution of Eq. (4) is:

$$l = \frac{(a + 1)l_0}{1 + al_0^{1+1/a}}, \quad \sin\theta = \frac{1 - l_0^{1+1/a}}{1 + al_0^{1+1/a}}. \quad (5)$$

Notice that the shape of the loop is a function of a single parameter a (for $a = 1$ the loop is a semicircle). For broken loops this parameter can be determined independently from our previous estimation of F_b and from the measured value of $\theta(s_b)$ at the breaking point $s = s_b$, $\theta(s_b) = 57^\circ \pm 6.6^\circ$. Equation (2) then yields $a = 1.36$. The shape calculated with this value matches nicely the observed ones [see Fig. 2(a)]. The value of a for

nonbroken loops, being proportional to the extension force, can then be deduced by a measurement of the peak of the PDL [$\sim 22 \mu\text{m}$ for the loops shown in Fig. 2(a) and $18 \mu\text{m}$ for the loops of Fig. 2(b)]. The shape thus calculated for a DNA loop on an antiDIG surface, with $a = 0.47$, is again in excellent agreement with the observation [see Fig. 2(b)].

In the previous discussion, we have considered the case where the force on the molecule was strong enough to elastically stretch it. There is another interesting regime, where the force on the molecule is balanced by its entropic (not bulk) elasticity. In that regime, the expected shape of the combed loops is very different from the one described above. Following Bustamante *et al.* [4], we adopt an approximate formula for the force vs extension of a stiff chain random polymer:

$$\frac{F_0 \xi}{k_B T} \sin\theta(l_0) = \frac{1}{4} \left(1 - \frac{l}{l_0}\right)^{-2} - \frac{1}{4} + \frac{l}{l_0}, \quad (6)$$

where ξ is the DNA persistence length. This scale invariant equation, which depends on one parameter $b \equiv F_0 \xi / k_B T$, can be solved. All its solutions possess a sharp tip: $\theta(0) < \pi/2$. We have not yet been able to reduce the tensile force on the molecule to observe that regime.

To summarize, our results are consistent with the following picture of the combing process. At the moving anchoring point of the molecule, i.e., at the transition between its combed dried portion and its part in solution a constant force is exerted on the molecule, whose magnitude F_0 or F_\perp depends on whether the part in solution is parallel or perpendicular to the meniscus. That force elastically stretches the molecule in the immediate vicinity of the meniscus, which is "glued" to the surface as the meniscus recedes.

We thank F. Heslot and A. Chiffaudel for their constant help and valuable criticism and J.P. Changeux for his support. A. S. is a U.S. NSF Post-Doctoral Fellow. A. B. is a Human Frontier Science Post-Doctoral Fellow. Laboratoire de Physique Statistique and Laboratoire de Physique de la Matière Condensée are laboratoires associés to the Centre National de la Recherche Scientifique and to the Universités Paris 6 and 7.

[1] M. Yanagida, Y. Hiraoka, and I. Katsura, Cold Spring Harbor Symp. Quant. Bio. **47**, 177 (1983).

[2] D.C. Schwartz and M. Koval, Nature (London) **338**, 520 (1989).

[3] W.D. Volkmuth and R.H. Austin, Nature (London) **358**, 600 (1992).

[4] S.B. Smith, L. Finzi, and C. Bustamante, Science **258**, 122 (1992); C. Bustamante, J.F. Marko, E.D. Siggia, and S. Smith, Science **265**, 1599 (1994).

[5] T.T. Perkins, D.E. Smith, and S. Chu, Science **264**, 819 (1994); T.T. Perkins, S.R. Quake, D.E. Smith, and S. Chu, *ibid.* **264**, 822 (1994).

[6] D.C. Schwartz *et al.*, Science **262**, 110 (1993).

[7] R.M. Zimmermann and E.C. Cox, Nucleic Acids Res. **22**, 492 (1994).

[8] A. Bensimon *et al.*, Science **265**, 2096 (1994).

[9] AntiDIG surfaces were made by successive coating of silanated surfaces with protein A and antidigoxigenine. DIG labeling of λ -DNA was done as follows: the right cos-end of λ -DNA was 3' end labeled with DIG-dUTP by terminal transferase and ligated to λ -DNA. Staining with YOYO1 was done according to the manufacturer's instructions.

[10] Three dilutions of bovine serum albumin (molecular weight 66 000) in phosphate buffer saline were used: 100, 10, and 1 $\mu\text{g}/\text{ml}$ 50 μl of each dilution was put on a silanated surface and covered with an untreated coverslip (diameter 1.8 cm), left to incubate at room temperature for about 1.5 h, and rinsed in water.

[11] Change of direction of the meniscus does not affect the combed portion of the molecule, and rehydration seems to leave the molecule bound to the surface.

[12] P.J. Hagerman, Annu. Rev. Biophys. Biophys. Chem. **17**, 265 (1988).

[13] M.E. Hogan and R.H. Austin, Nature (London) **329**, 263 (1987).

[14] The hydrodynamic drag on a molecule of length $L = 100 \mu\text{m}$ in a typical flow of $10 \mu\text{m}/\text{sec}$ is $\sim \eta L v \sim 1 \text{ pN}$, where $\eta = 10^{-2} \text{ P}$ is the viscosity of water.

[15] E.L. Florin, V.T. Moy, and H.E. Gaub, Science **264**, 415 (1994); G.U. Lee, D.A. Kidwell, and R.J. Colton, Langmuir **10**, 354 (1994).

[16] I. Parra and B. Windle, Nature Genetics **5**, 17–21 (1993).

[17] A. Stasiak and E. Di Capua, Nature (London) **299**, 185 (1982).

[18] The order of magnitude of the forces (a few hundred pN) is roughly what one expects from the surface tension on a wire with the diameter of DNA: $\pi \gamma D \sim 400 \text{ pN}$. ($\gamma = 0.072 \text{ N/m}$ is the surface tension of the air/water interface.)

[19] R.E. Harrington and B.H. Zimm, J. Phys. Chem. **69**, 161 (1965).

[20] Notice that F_0 might not be equal to F_\perp , since the molecule in solution is parallel to the interface in one case, perpendicular to it in the other.