pH-Dependent Specific Binding and Combing of DNA

J.-F. Allemand,* D. Bensimon,* L. Jullien,# A. Bensimon,§ and V. Croquette*
§Laboratoire de Biophysique de l'ADN, Institut Pasteur, 75724 Paris Cedex 15, #Département de Chimie, ENS, URA 1679 CNRS, 75231 Paris Cedex 05; and *LPS, ENS, URA D 1306 CNRS, associé aux universités Paris VI et Paris VII, 75231 Paris Cedex 05, France

ABSTRACT Recent developments in the rapid sequencing, mapping, and analysis of DNA rely on the specific binding of DNA to specially treated surfaces. We show here that specific binding of DNA via its unmodified extremities can be achieved on a great variety of surfaces by a judicious choice of the pH. On hydrophobic surfaces the best binding efficiency is reached at a pH of ~5.5. At that pH a ~40-kbp DNA is 10 times more likely to bind by an extremity than by a midsegment. A model is proposed to account for the differential adsorption of the molecule extremities and midsection as a function of pH. The pH-dependent specific binding can be used to align anchored DNA molecules by a receding meniscus, a process called molecular combing. The resulting properties of the combed molecules will be discussed.

INTRODUCTION

The ability to specifically bind DNA to a surface by its extremities has found many useful applications, from gene mapping, sequencing, and analysis (Chee et al., 1996; Meng et al., 1995), via the development of very sensitive immunological assays (immuno-PCR) (Sano et al., 1992), to biophysical studies of single molecules (Strick et al., 1996; Cluzel et al., 1996; Smith et al., 1996). Most of these applications achieve the required binding specificity via specific biochemical reactions between a (possibly modified) DNA molecule and an appropriately treated surface. For example, the extremity of the molecule can be functionalized with biotin and bound specifically to streptavidin-coated surfaces (Strick et al., 1996; Cluzel et al., 1996; Smith et al., 1996). Similarly, surfaces coated with oligonucleotides can be used to recognize the complementary segment of DNA molecules and detect sequence polymorphism (Chee et al., 1996). We have recently discovered a new, physicochemical way of achieving a very specific binding of DNA via its extremities without any modification of the molecule. This specific binding was observed at pH 5.5 (MES (2-[N-morpholino]ethanesulfonic acid) buffer) on surfaces coated with a silane possessing a vinyl ($-CH{=}CH_2$) end group (Bensimon et al., 1994). The strength of the bond is sufficient to withstand the capillary forces (typically >160 pN) that stretch the molecule to 150% of its crystallographic length when the solvent/air interface recedes (for example, through evaporation). We have shown that this process (called molecular combing; see Fig. 1) can be used to align DNA molecules for the localization of DNA markers along a single molecule by fluorescence hybridization (Weier et al., 1995; Michalet et al., 1997).

Here we show that specific binding of DNA via its extremities can be achieved on a great variety of surfaces (not only ones silanized with a vinyl end group) by a judicious choice of pH.

MATERIALS AND METHODS

Surface preparation

Surfaces coated with a vinyl-silane (C=C surfaces)

Glass surfaces are cleaned in one-third hydrogen peroxide (30%), two-thirds ammonium hydroxide (30%), boiled, and rinsed in distilled water. They are introduced into an hermetic glass chamber filled with O_2 and further cleaned for 1 h in the ozone atmosphere produced by UV illumination of O_2. The chamber is flushed with N_2 and then filled with N_2/10% H_2O for 20 min. The chamber is again flushed with N_2 for 10 min, and 100 μl of octyltrichlorosilane ($CH_2{=}CH-(CH_2)_6-SiCl_3$) is added and left to incubate overnight. Surfaces can be stored at room temperature until used.

Polystyrene surfaces

Polystyrene (MW 280,000 or 50,000) is dissolved in toluene at a concentration of 50 or 100 mg/ml. The solution is deposited on a glass coverslip and spin-coated for 30 s at 3000 rpm. Neither the polystyrene molecular weight nor the rotation speed of spin-coating affects the results. For the first trials, pieces of Petri boxes were dissolved in toluene and the glass coverslips were immersed in that solution for a few seconds. These polystyrene-coated surfaces and those silanized with a vinyl group yield the most reproducible results.

Polymethylmetacrylate surfaces

A solution of 0.5 mg/ml polymethylmetacrylate (PMMA) (MW 300,000) is spin-coated under the same conditions as polystyrene. The surfaces are then annealed for half an hour at 150°C.

Clean glass

Glass coverslips are cleaned for 30 min in a solution consisting of one-third hydrogen peroxide (30%) and two-thirds sulfuric acid (18 M), rinsed in distilled water, left for 10 min in boiling distilled water, dried with an argon flow, and then immediately used.

Received for publication 5 March 1997 and in final form 23 June 1997.

Address reprint requests to Dr. Aaron Bensimon, Laboratoire de Biophysique de l'ADN, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris cedex 15, France. Tel.: 33-10-40-61-32-40; Fax: 33-10-45-68-87-90; E-mail: abensim@pasteur.fr.

0006-3495/97/10/2064/07 $2.00

FIGURES

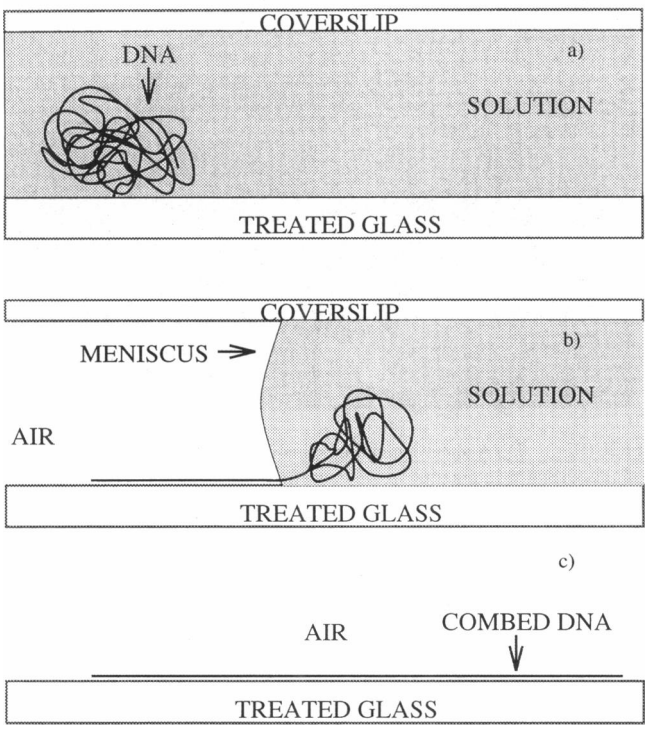

FIGURE 1 Principle of molecular combing. (*a*) DNA incubated at an adequate pH on a treated surface spontaneously binds by one (or two) extremities to the surface. (*b*) As the interface moves, the DNA is stretched perpendicular to the receding meniscus, as are algae by the receding tide. (*c*) It is left linearized and dry behind the meniscus.

Polylysine and polyhystidine surfaces

Glass coverslips are cleaned as previously described and then immersed for 5 min in 10 M NaOH. After being washed in pure water, they are immediately immersed into a 1–500 μg/ml aqueous solution of polypeptide (with a MW of 15,000–50,000) for 2 min and then dried with an argon flow.

Because of the variability of the glass surface (even after cleaning), the reproducibility of the modified surfaces remained a problem. The binding pH varied from one batch to another, and even between the different surfaces of one batch. Commercial surfaces (Polylysine, Menzel-Glaser) also allowed combing, but were even less reproducible.

Amino-silane-coated surfaces

The glass is cleaned as described before. The clean coverslip is immersed in a methanol solution containing 2.5–5 mg/ml of γ-amino-triethoxysilane ($NH_2 - (CH_2)_3 - Si(OCH_2CH_3)_3$). The surfaces are pulled out of the solution (to stop the reaction), washed first in pure water and then ethanol, and finally dried with an argon flow.

DNA staining

To visualize DNA, we prestrained it with YOYO1 (Molecular Probes, Eugene, OR) at a ratio of 1 dye to 20 bases. The staining procedure is the one proposed by the manufacturer. Staining can also be done after combing, implying that the dye does not significantly affect the phenomenon. As uniform staining after combing is a bit more complicated to achieve, all of the experiments presented here were performed with prestained DNA.

Combing technique

See Fig. 1. In some of the experiments reported here, molecular combing is achieved by the evaporation, between two coverslips, of a deposited droplet, as previously described (Bensimon et al., 1994, 1995). Alternatively, we use a method inspired by the Langmuir-Blodgett deposition technique that we call dynamic molecular combing (see Michalet et al., 1997). The surface is incubated in a DNA solution at the right pH and then pulled out slowly. This technique has an advantage over the evaporation method: because the surfaces are incubated in a rather large volume (2–20 ml) of buffer solution, modification of the pH by dissolution of CO_2 is less of a problem. Considering the narrow pH range in which molecular combing is observed, this effect could be important in droplets of volumes as small as 5 μl. The Langmuir-Blodgett technique also has other advantages (see Michalet et al., 1997).

RESULTS AND DISCUSSION

DNA was specifically bound to several different surfaces: hydrophobic surfaces (graphite, Teflon (oriented (Wittmann and Smith, 1991) or not), glass coated with vinyl-silanes, polystyrene, polydimethylsiloxane (PDMS) (Deruelle, 1996) (even transparency sheets) or hydrophilic surfaces (cleaned glass or glass coated with amino-silanes, polylysine, polyhistidine, or polymethylmetacrylate). On all of these surfaces the same behavior is observed: at low pH, DNA molecules adsorb strongly and nonspecifically (i.e., they are not fluctuating in solution and are not extended by a receding meniscus), whereas at a high pH they adsorb very weakly (i.e., dragged by a receding meniscus) or not at all. In between there exists a narrow pH range where DNA binds to the surface strongly and specifically by its extremities (see Fig. 2).

The pH range where strong nonspecific adsorption of DNA is observed (see Fig. 3) is commonly used for chromosome spreading (Parra and Windle, 1993) on various surfaces (Hu et al., 1996), particularly on polylysine-coated ones (Meng et al., 1995). Molecular combing should not be confused with these techniques. They rely on an uncontrolled flow to stretch the molecules as they adsorb on the surface and yield nonreproducible results: the absorbed molecules are unevenly stretched and aligned and are often broken. Molecular combing does not require a shear flow. It relies on a receding meniscus to stretch DNA spontaneously bound to the surface by its extremities (see Fig. 1) (Bensimon et al., 1994). This occurs in a very narrow range of pH (typically ~0.2 units on hydrophobic surfaces: polystyrene, C=C surfaces, Teflon, graphite, etc.), with the best binding pH varying slightly with surface treatment (see Fig. 4).

On hydrophobic surfaces the optimal pH for binding of DNA by its extremities is found near ~5.5. On surfaces coated with ionizable groups (clean glass, or glass coated with polylysine, polyhistidine, or amino-silanes), the optimal pH is apparently linked to the surface charge and thus is linked to the pK_a of the surface groups and their density (see Fig. 5). As can be seen in Fig. 4, the specific binding

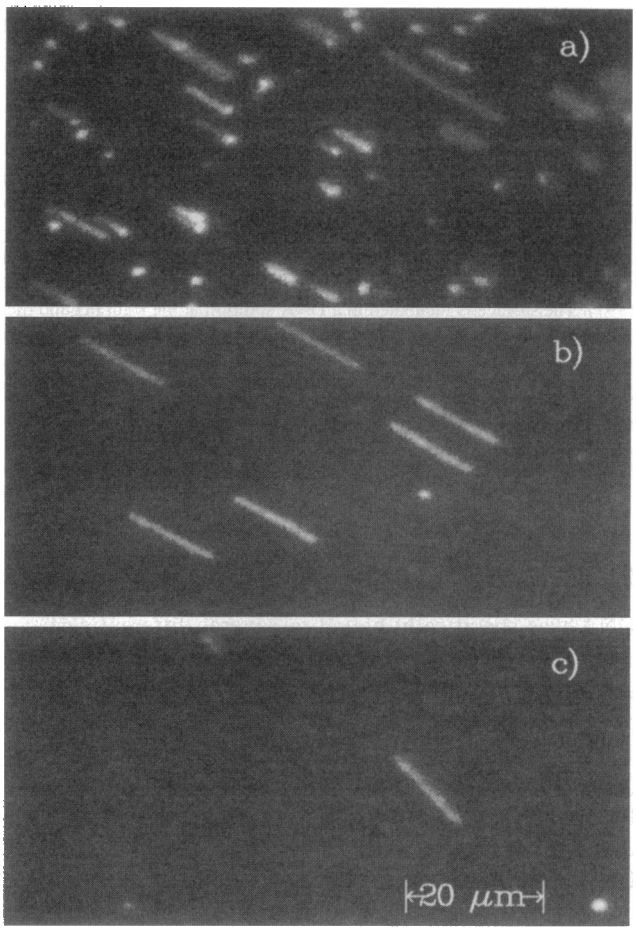

FIGURE 2 Images of adsorbed and combed DNA molecules on different surfaces and at different pHs. The molecules are visualized with YOYO1. (*a*) Polystyrene at pH 4.2. The molecules appear as bright spots; only a few are nonevenly stretched. (*b*) Polystyrene at pH 5.5. Many molecules are combed. (*c*) Polystyrene at pH 6.5. Only a few molecules were combed. The DNA solution concentration is the same in *b* and *c*.

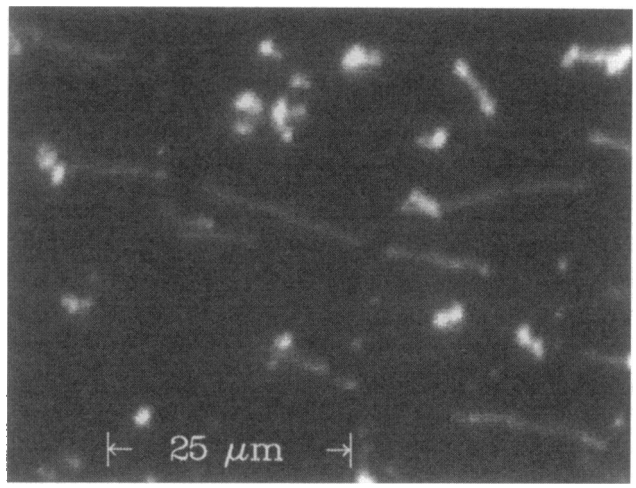

FIGURE 3 DNA shear spreading. This image shows the general aspect of the strongly interacting regime on amine-coated surfaces at low pH (~6). The bright spots are DNA molecules like the ones seen in Fig. 2 *a*. The extended molecules are stretched by a shear flow as the droplet containing DNA molecules spreads. Notice the different directions and uneven stretching, in contrast with the uniform extension observed for combed molecules in Fig. 2 *b*.

efficiency is a very sensitive function of the pH. PMMA is the only surface type so far where we have observed molecular combing over a large pH range. The large chemical diversity of the surfaces studied and the similar behavior of their interactions with DNA under varying pH imply that no chemical reaction is involved in the binding between the surface and the DNA ends.

To quantify the binding specificity, we compared the anchoring of a supercoiled ~40-kb plasmid and its linearized form (gift of O. Hyrien, I. Lucas, and C. Maric) on polystyrene at pH ~5.5 (and the subsequent combing) (see Fig. 6). Combed linear molecules were 10 times more numerous than circular ones (their binding is probably due to residual nonspecific adsorption). We thus conclude that DNA anchoring by its extremities is 10 times more probable, at that pH, than is anchoring by a midsegment. The relative extension (19% or 8.5 μm) of the circular plasmid is observed to be half the relative extension (40% or 20 μm) of the linearized form. This implies that the capillary force pulling on the two double-strand fibers is the same and not twice the force pulling on a single double-strand molecule.

Once the molecules have specifically bound at the optimal pH (e.g., 5.5 on hydrophobic surfaces), they remain so even if the pH is raised to the noninteracting range (e.g., 7 or 9). This result suggests a very simple way to bind unmodified double-stranded DNA by its extremities on hydrophobic surfaces. Moreover, this bond is strong enough to withstand the capillary force acting on the DNA as the meniscus recedes. Rehydration after combing may induce partial desorption if performed when the surface is not perfectly dry (a very thin film of water may remain).

The previous results suggest that the transition between adsorption and nonadsorption occurs at a different pH for the extremities and the inner parts of the molecules. It is shifted to lower pH for the midsection compared to the DNA's extremities. In particular, there is a (narrow) pH range where the midsection does not adsorb, whereas the extremities can still bind to the surface. To account for this behavior, we would like to propose two models, depending on the presence or absence of electrostatic charges on the surface.

On surfaces coated with ionizable groups, at a pH that lies below the pK_a characterizing the basicity of the surface groups (e.g., amines), protonation takes place (e.g., $-NH_2 \rightarrow -NH_3^+$). The surface charge becomes positive and is a decreasing function of pH. It also strongly depends on ionic strength and on coverage density by basic groups. At low pH, the electrostatic attraction between the oppositely charged surface and DNA dominates. The molecules are observed to adhere strongly and nonspecifically to the surface. At a high pH ($>pK_a$), the absence of protonation means that the surface is neutral. However, it is known that

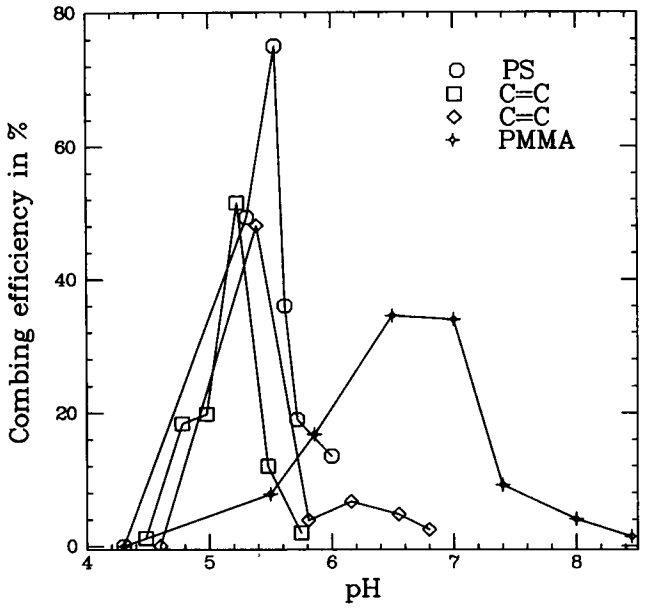

FIGURE 4 The combing efficiency (defined as the number of combed molecules divided by the number of spots one can see at low pH, where DNA is strongly adsorbed on the surface) on three different kinds of surfaces: silanized with a vinyl group (from two different batches), and spin-coated with polymethylmetacrylate or polystyrene. The very hydrophobic surfaces have an optimal combing efficiency at pH ~5.5 (50 mM MES buffer) with a very narrow peak. On PMMA-coated surfaces, molecules were combed between pH 6.5 and 7.5, with an optimum at pH ~7 (bis-tris-propane, 50 mM buffer). On some batches the peak could be shifted to lower pH, but still remained large compared to hydrophobic surfaces.

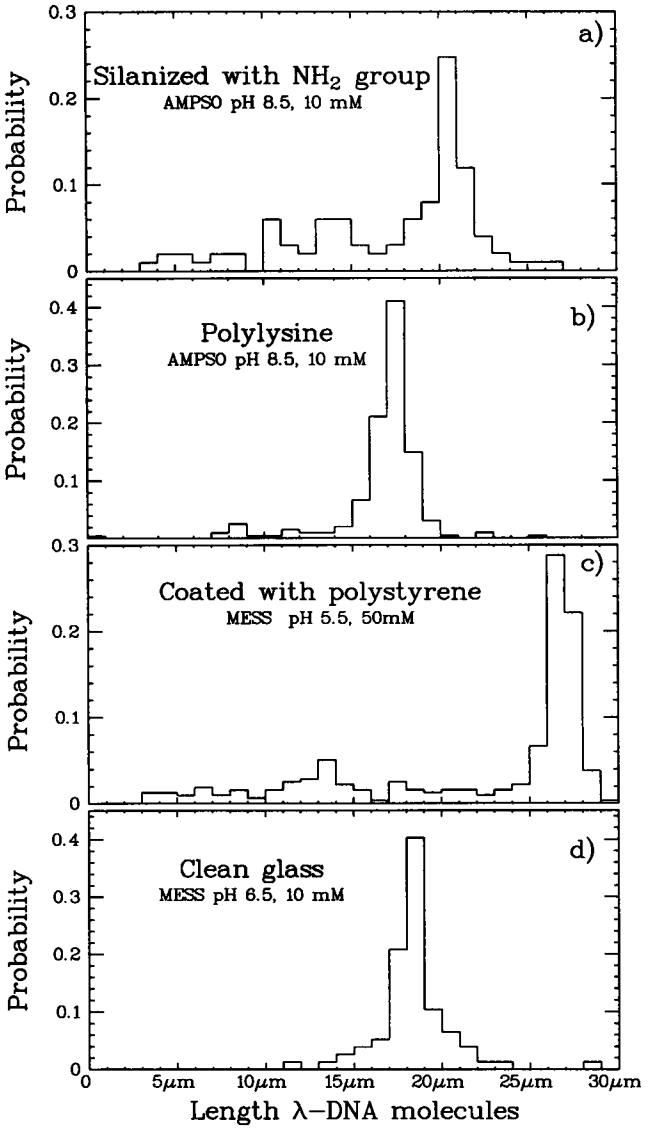

FIGURE 5 Histograms showing the length of λ-DNA molecules (crystallographic length 16.2 μm) combed on different surfaces. The length of the stretched molecules is greater on hydrophobic surfaces. Thus the stretching force applied by the meniscus is linked to the hydrophobicity of the surface, as previously observed (Bensimon et al., 1995). Surfaces coated with polystyrene are very hydrophobic, and consequently the combed molecules are more extended on these surfaces.

in a high dielectric medium (such as water), a charged object near a low dielectric surface (glass) experiences a repulsive force, as if an image charge of the same sign existed on the other side of the surface (Jackson, 1962). Hence the charged DNA molecule near a neutral glass surface is nonetheless repelled by its image charge. The crossover between strong adsorption and repulsion happens at such a pH that the electrostatic attraction with the surface is balanced by the repulsion between the DNA and its image charge. As demonstrated by the work of Stein et al. (1995), the DNA's extremities are more negative than its midsegment. Thus the interaction with the surface (including image charge effects) will not be the same for the ends as for the rest of the molecule. Experimentally we do observe a narrow pH range where the DNA's extremities still bind, whereas its inner parts no longer adhere. This electrostatic hypothesis is further supported by the following observation (see Fig. 7). On a surface coated partly with negatively charged groups (polyglutamic acid) and partly with positively charged ones (polylysine), one observes, at pH ~6, a strong nonspecific adsorption of DNA on the polylysine-coated part, but no adsorption on the polyglutamine-coated one. Because the specific binding pH is sensitive to the surface charge density (which is difficult to control), reproducible combing results for these positively charged surfaces are more difficult to obtain than for hydrophobic ones. Recent results by another group (Yokota et al., 1997) confirm these difficulties (to control the electrostatic interaction, these authors use precoating with single-stranded DNA; we conjecture that they could use polyglutamic acid in the same way).

On neutral hydrophobic surfaces, a different mechanism might be responsible for the specific binding of the DNA molecules. At low pH, the DNA bases undergo intensive protonation. The protonation depends on pH and ionic strength: it is half complete at pH ~3.0 in 0.1 M salt (Bunville et al., 1965) and at pH ~3.7 in 0.02 M (Zimmer et al., 1968). In low salt, it is already equal to a few percent

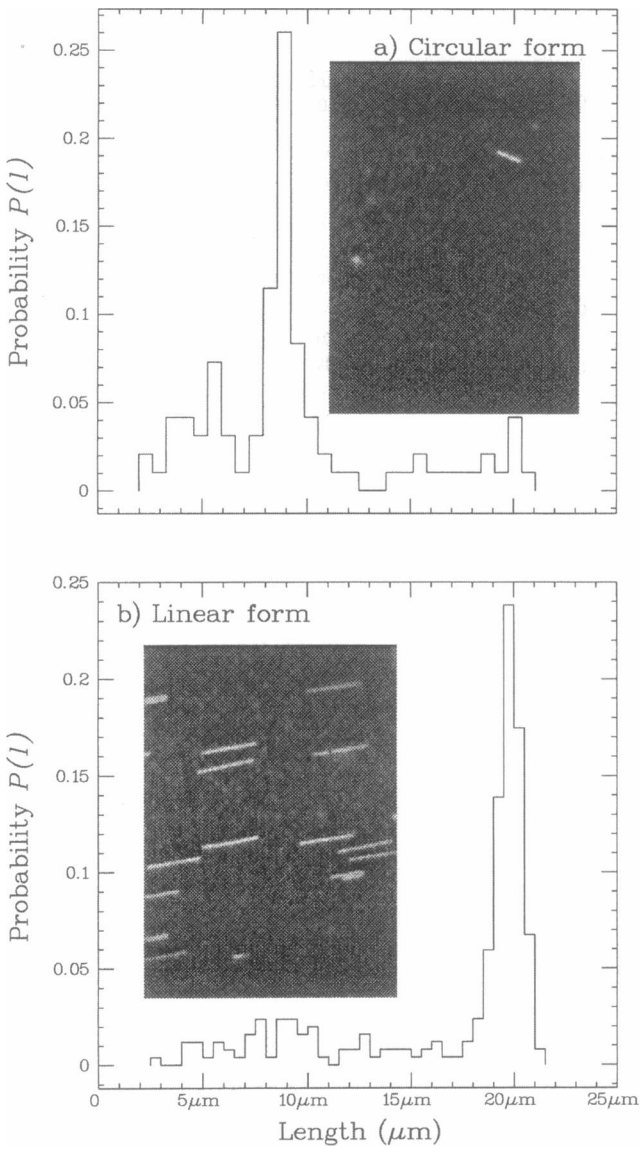

FIGURE 6 Differential adsorption of a 42-kb DNA in its supercoiled (*a*) and linear (*b*) forms. The linear form is obtained from the digestion of the supercoiled plasmid by *Eco*RI. The combing was performed at pH 5.5 in 50 *mM* MES on polystyrene surfaces. As can be seen in the images, combed linear molecules are 10 times more numerous than combed circular DNA. Thus binding by the extremities of the molecule is much more probable than binding by a midsegment.

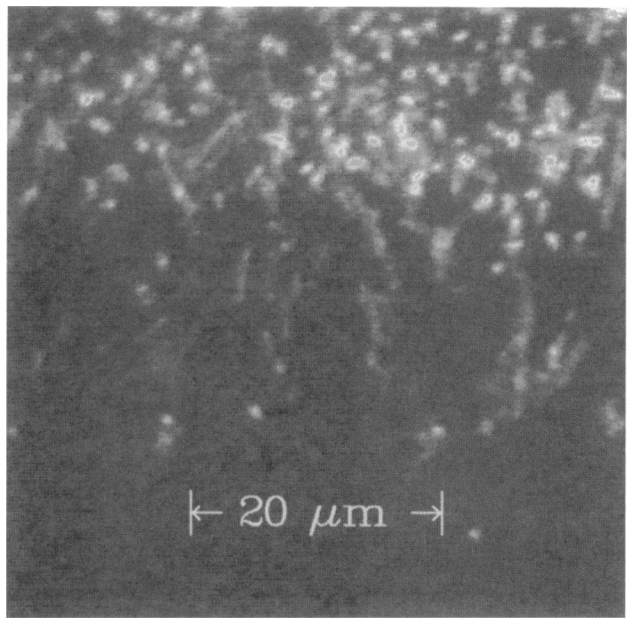

FIGURE 7 DNA adsorption on a polylysine surface with one part (the bottom) previously immersed in polyglutamic acid. At pH ~6, the polylysine part is positively charged and the polyglutamic-coated one is negatively charged. The stained DNA is strongly adsorbed and does not fluctuate on the polylysine side (the bright region), and no adsorption is observed at all on the polyglutamic side. Between the two regions, some molecules attached by both extremities are seen fluctuating in solution.

at pH ~5.5 (where specific DNA binding is observed). That protonation induces a DNA melting that exposes the hydrophobic core of the helix. The affinity between the molecule and the hydrophobic surface (Belotserkovskii and Johnston, 1996) is thus enhanced, which accounts for the strong nonspecific adsorption at low pH. As the pH is increased, denaturation gradually disappears and becomes restricted to the DNA's extremities (Benight and Wartell, 1983), as suggested by the dependence of potentiometric curves (Bunville et al., 1965) and melting temperatures (Belotserkovskii and Johnston, 1996) on DNA length. Under these conditions (pH ~5.5), the affinity between the surface and the molecule is essentially specific to its extremities. Finally, at pH greater than 6, little DNA denaturation takes place. The molecule is repelled by its image charge. The unusual behavior on PMMA (a broad rather than narrow pH range over which specific end binding is observed) may result from additional contributions favoring DNA/surface interactions at a higher pH. For instance, carboxyl groups on PMMA might form hydrogen bonds with the DNA bases, stabilizing the denaturated extremities.

The degree of extension of the DNA (the increase in the length of the molecule relative to its crystallographic size) during combing depends on the surface treatment (Bensimon et al., 1995). Fig. 5 shows length histograms of λ-DNA (crystallographic length 16.2 μm) stained with YOYO1 and combed on various surfaces. As previously noticed (Bensimon et al., 1995), on hydrophilic surfaces (e.g., cleaned glass or polylysine) the degree of extension of the molecule (approximately equal to the force pulling on it) is small (<25%), and the deduced capillary force on the molecule is weak (>100 pN). On hydrophobic surfaces (e.g., coated with polystyrene) the relative extension is larger and thus the force is stronger. It is worth noting that we have not been able to visualize combed molecules on all of the surfaces that specifically bind to the DNA ends. The specific binding can be checked by stretching the molecules in solution with a flow. However, on some surfaces (e.g., graphite) the fluorescence of the staining dye (YOYO1, POPO1, or YOYO3) is quenched in the dry conditions left behind the meniscus. Because the bound molecules were not seen to be

dragged by the receding interface, we assume that they are combed on these surfaces as on others, where the stained, stretched, and dried molecules are still fluorescent. It is worth noting that DNA can be combed unstained and then stained after combing (this usually also increases background fluorescence) with a similar extension (for the effect of the ratio dye per base pair on the extension of the combed molecules, see also Bensimon et al., 1995).

Some of the properties of the molecular combing process are worth recalling. The action of the meniscus on the molecule is localized in the immediate vicinity of the interface. In front of it the molecule in solution is fluctuating freely. Behind, the molecule sticks to the surface and is insensitive to changes in the orientation of the meniscus. That property has the very important consequence that the stretching of a molecule anchored at one end is uniform. This is demonstrated in Fig. 8, which shows the extension of molecules as a function of their number of base pairs on various surfaces (C═C and polystyrene). As can be seen, the extension of the molecules is proportional to their number of base pairs, as expected for homogeneous stretching. It also suggests that the stretching is sequence independent, which is reasonable for the large forces involved (>100 *pN*; see Bensimon et al., 1995). This result is consistent with those from the literature (Weier et al., 1995), even if some of the images shown in this article seem to be due to shear spreading in the strongly interacting phase, rather than combing at the optimal pH range (Yokota et al., 1997; Michalet et al., 1997).

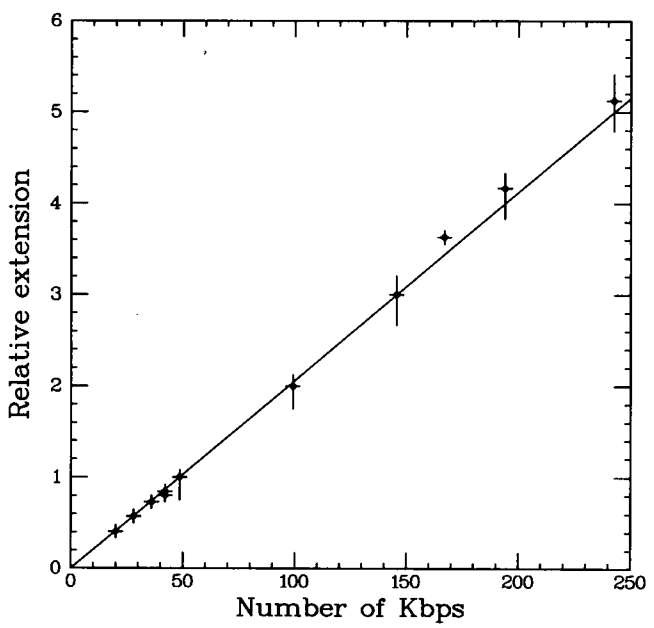

FIGURE 8 Different sources of DNA of various lengths (20, 28, 36, 42 (all provided by I. Lucas, O. Hyrien, C. Maric), 48.5 (λ-DNA), 167 (T4-DNA) kb, and a mix of λ multimers) were combed on different types of surfaces (polystyrene and C═C). All of the lengths were rescaled by the λ extension on each type of surface (from 24 to 27 μm) to compare the relative extensions on different surfaces. The linearity of the experimental points is in good agreement with what is expected for a uniform extension. Hence the relative extension does not depend on DNA length or DNA sequence. This fact provides an easy way to measure DNA length and is important for applications in mapping. The uncertainty comes mostly from the breaking of the molecules during manipulations before combing (direct visualization of the receding meniscus showed that the molecules were not broken during the combing process), but also from length measurement and small variabilities in surface characteristics. Combing has been achieved with the DNAs presented here, but also with T5 DNA, YACs, BACs, λ-DNA cut with *NruI* (which provides blunt extremities), and what is more important for applications, with genomic DNA (see Michalet et al., 1997).

CONCLUSION

These results demonstrate that specific binding of DNA by its extremities on various surfaces is a physical process controlled by the pH of the solution, the optimization of which is surface dependent. The subsequent molecular combing process is thus a very general phenomenon that results in the alignment of uniformly stretched DNA molecules. It does not require any modification of the molecule, it is gentle for the molecules, and it is easy to perform on a great variety of surfaces. The recognition of the significance of the pH should facilitate the use of molecular combing for gene mapping and diagnostics.

We thank C. Schurra and T. R. Strick for preparing the C═C surfaces; O. Hyrien, I. Lucas, C. Maric for providing plasmids of different lengths in their circular and linear forms; F. Ladan for introducing us to spin-coating; V. Bouchiat for providing PMMA; H. Hervet for providing the PDMS surfaces; J. C. Wittmann for giving us oriented Teflon surfaces; and J. F. Joanny for reminding us of the image charge. We also thank them for useful discussions.

We acknowledge the support of the "Association Française contre les Myopathies" (AFM), "Groupement de Recherche et d'Etudes sur les Génomes" (GREG), C.N.R.S., Universités Paris 6 and Paris 7, and Ecole Normale Supérieure.

REFERENCES

Belotserkovskii, B. P., and B. H. Johnston. 1996. Polypropylene tube surfaces may induce denaturation and multimerization of DNA. *Science.* 271:222.

Benight, A., and R. Wartell. 1983. Influence of base-pair changes and cooperativity parameters on the melting curves of short DNAs. *Biopolymers.* 22:1409–1425.

Bensimon, A., A. Simon, A. Chiffaudel, V. Croquette, F. Heslot, and D. Bensimon. 1994. Alignment and sensitive detection of DNA by moving interface. *Science.* 265:2096–2098.

Bensimon, D., A. Simon, V. Croquette, and A. Bensimon. 1995. Stretching DNA with a receding meniscus: experiments and models. *Phys. Rev. Lett.* 74:4754–4757.

Bunville, L., E. Geiduschek, M. Rawitscher, and J. Sturtevant. 1965. Kinetics and equilibria in the acid denaturation of deoxyribonucleic acids from various sources. *Biopolymers.* 3:213–240.

Chee, M., R. Yang, E. Hubbell, A. Berno, X. Huang, D. Stern, J. Winkler, D. J. Lockhart, M. S. Morris, and S. P. A. Fodor. 1996. Accessing genetic information with high-density DNA arrays. *Science.* 274:610–614.

Cluzel, P., A. Lebrun, C. Heller, R. Lavery, J.-L. Viovy, D. Chatenay, and F. Caron. 1996. DNA: an extensible molecule. *Science.* 271:792–794.

Deruelle, M. 1996. Les polymeres aux interfaces: application a l'adhesion solide elastomere. Ph.D. thesis. Université Pierre et Marie Curie, Paris.

Hu, J., M. Wang, H.-U. G. Weier, P. Frantz, W. Kolbe, D. Ogletree, and M. Salmeron. 1996. Imaging of single extended DNA molecules on flat

(aminopropyl)triethoxysilane-mica by atomic force microscopy. *Langmuir.* 12:1697–1700.

Jackson, J. 1962. Classical Electrodynamics. Wiley, New York.

Meng, X., K. Benson, K. Chada, E. Huff, and D. Schwartz. 1995. Optical mapping of lambda bacteriophage clones using restriction endonucleases. *Nature Genet.* 9:432–438.

Michalet, X., R. Ekong, F. Fougerousse, S. Rousseaux, C. Schurra, S. Povey, J. S. Beckmann, A. Bensimon, N. Hornigold, M. van Slegtenhorst, and J. Wolfe. 1997. Dynamic molecular combing: stretching the whole human genome for high resolution studies. DNA. *Science.* (in press).

Parra, I., and B. Windle. 1993. High resolution visual mapping of stretched DNA by fluorescent hybridization. *Nature Genet.* 5:17–21.

Sano, T., C. L. Smith, and C. R. Cantor. 1992. Immuno-pcr: very sensitive antigen detection by means of specific antibody-DNA conjugates. *Science.* 258:120–122.

Smith, S., Y. Cui, and C. Bustamante. 1996. Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. *Science.* 271:795–799.

Stein, V. M., J. P. Bond, M. W. Capp, C. F. Anderson, and M. R. Anderson, Jr. 1995. Importance of coulombic end effects on cation accumulation near oligonucleotide B-DNA using Na NMR. *Biophys. J.* 68: 1063–1071.

Strick, T., J. Allemand, D. Bensimon, A. Bensimon, and V. Croquette. 1996. The elasticity of a single supercoiled DNA molecule. *Science.* 271:1835–1837.

Weier, H.-U. G., M. Wang, J. Mullikin, Y. Zhu, J.-F. Cheng, K. Greulich, A. Bensimon, and J. Gray. 1995. Quantitative DNA fiber mapping. *Hum. Mol. Genet.* 4:1903–1910.

Wittmann, J., and P. Smith. 1991. Highly oriented thin films of PTFE as a substrate for oriented growth of materials. *Nature.* 352:414–417.

Yokota, H., F. Johnson, H. Lu, R. Maxwell Robinson, A. Belu, M. Garrison, B. Ratner, B. Trask, and D. Miller. 1997. A new method for straightening DNA molecules for optical restriction mapping. *Nucleic Acids Res.* 25:1064–1070.

Zimmer, C., G. Luck, H. Venner, and J. Fric. 1968. Studies on the conformation of protonated DNA. *Biopolymers.* 6:563–574.