Journal of Muscle Research and Cell Motility **23**: 367–375, 2002.
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Mechanics and imaging of single DNA molecules

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Abstract

We review recent experiments that have revealed mechanical properties of single DNA molecules using advanced manipulation and force sensing techniques (scanning force microscopy (SFM), optical or magnetic tweezers, microneedles). From such measurements, intrinsic relevant parameters (persistence length, stretch modulus) as well as their dependence on external parameters (non-physiological conditions, coating with binding agents or proteins) are obtained on a single-molecule level. In addition, imaging of DNA molecules using SFM is presented.

Introduction

The study of nucleic-acid structure has experienced a fundamental shift toward methods that are capable of studying the physical properties of single molecules. During the last 10 years several force-sensitive experiments have been used to stretch and manipulate single DNA molecules. The application of hydrodynamic drag (Bensimon *et al.*, 1995; Perkins *et al.*, 1997; Otobe and Ohani, 2001), magnetic tweezers (Smith *et al.*, 1992; Strick *et al.*, 2000a), glass needles (Leger *et al.*, 1999), optical tweezers (OT) (Smith *et al.*, 1996; Bouchiat *et al.*, 1999; Hegner *et al.*, 1999) and scanning force microscopy (SFM) (Rief *et al.*, 1999) allowed to investigate the mechanical response of single DNA molecules. For all of these force-sensitive experiments, the following requirements have to be fulfilled. First, the molecule has to be tethered in-between two microscopic objects whose position can be precisely defined (nm accuracy). Second, the force acting on moving parts has to be quantified with force resolution in the picoNewton (pN) regime. Force spectroscopy on single molecules is thus technically most demanding. One can select a method of generating tension that is tailored to a specific application for a specific range of forces. Today, the magnetic beads and the OT technique are best suited to measure the entropic and enthalpic force arising in single-molecule DNA experiments. In particular, magnetic tweezers are sensitive in the range of 0.01–10 pN and OTs have sensitivity in a range of 0.1–200 pN (Grange *et al.*, 2002). The force limit (0.1 pN) of OT is two orders of magnitudes lower than in conventional SFM based techniques. The largest stretching forces (10–10,000 pN) are generated by SFM, in which the molecule of interest is stretched between a surface and a cantilever. For each of these methods the applied force can be continuously and accurately varied; thus, the energies of various nucleic acid and nucleic acid – protein structural transitions can be quantified.

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In this article we focus on recent experiments performed on single DNA molecules using the techniques mentioned above. In particular, we discuss mechanical properties and stress-induced transitions of a DNA single molecule when stretched beyond its entropic regime under both physiological and non-physiological conditions.

This review is organized as follows. In the first part we briefly mention simple theoretical models often used to describe the behavior of a polymer either at low (entropic regime) or high (enthalpic regime) force. Anchoring the molecules is certainly a key point in single-molecule measurements, which determines the success of the experiment. This is discussed in details in a second part. Then, we present typical force vs. extension curves obtained either on dsDNA, ssDNA, and discuss how external parameters (salt, surrounding proteins, etc.) can affect the observed mechanical properties. We also briefly mention experiments performed to twist, unzip or unbind single DNA molecules. Finally, recent measurements on DNA imaging are highlighted.

Models for DNA elasticity

The relevance of single-molecule force experiments lies in the fact that important parameters that describe the stiffness of the polymer (e.g. DNA) can be extracted. Such parameters are not accessible when measurements are performed on an ensemble, simply because this would need the synchronization of all stretching events. Again, we emphasize that such stretching experiments (force vs. distance) need to have a very high-force sensitivity. To give order of magnitudes, the characteristic force required to align a polymer made of identical rigid units of length b is $\sim kT/b$ and is only about 0.1 pN for dsDNA.

Entropic elasticity

Depending on the polymer different models are applied to correctly describe its entropic elastic behavior

(Merkel, 2001). The freely jointed chain (FJC) model (Flory,1969) considers a chain of identical N rigid subunits that are freely able to rotate (i.e. the angle between the different segments is not fixed):

$$\frac{x}{L} = \coth\left(\frac{Fb}{k_B T}\right) - \frac{k_B T}{Fb} \quad (1)$$

where x is end-to-end length, L is the contour length of the polymer ($L=Nb$), and b denotes the Kuhn length. Alternatively, the wormlike chain model (WLC) can be used (Bustamante *et al.*, 1994; Marko and Siggia, 1995; Rivetti *et al.*, 1998). In the WLC model, the polymer is treated as an uniform flexible rod and bending costs energy. In this case, the force-extension relation reads:

$$\frac{FA}{k_B T} = \frac{1}{4}\left(1 - \frac{x}{L}\right)^{-2} + \frac{x}{L} - \frac{1}{4} \quad (2)$$

where A is known as the persistence length (the distance along which the molecule can be considered as rigid). Note that (i) Equation (2) is only an approximation to the WLC model and additional correction terms should be added for a more rigorous treatment (Bouchiat *et al.*, 1999) (ii) the persistence length A is defined to be half of the Kuhn length b.

Enthalpic elasticity

At higher forces (above 5–10 pN for DNA), the elastic response of the polymer under an applied tension is not more purely entropic. In this case, enthaplic contributions have to be taken into account (Odijk, 1995) and experimental data are usually fitted using an extensible WLC model (we consider that – due to some stretchability – the contour length linearly increases with the applied force) (Smith *et al.*, 1996):

$$\frac{x}{L} = 1 - \frac{1}{2}\left(\frac{k_B T}{FA}\right)^{1/2} + \frac{F}{S} \quad (3)$$

where S denotes the stretch modulus of the polymer. Alternatively, models were the Kuhn segments are able to stretched have been proposed (Smith *et al.*, 1996).

Immobilization of molecules to surfaces

A challenge in single-molecule experiments is to specifically and strongly attach molecules onto surfaces. In this review, we focus on DNA single molecules although the techniques presented in this section might be applied to other biological samples. Different procedures exist depending on the requirements whether (i) a covalent, a non-covalent, or a non-specific coupling has to be achieved (ii), or a SFM or an optical (magnetic) tweezers experimental setup is used.

Covalent coupling – although it involves more steps and is somehow more difficult to achieve – is always best suited due to its high strength. In SFM experiments dealing with short molecules, single thiol-modified ssDNA molecules were covalently attached to an activated glass surface (silanized with an aminopropyltriethoxysilane) through a poly(ethylene glycol)-α-maleimide-ω-*N*-hydroxysuccinimide-ester spacer (Chrisey *et al.*, 1996; Strunz *et al.*, 1999). Both the glass and the tip surfaces were treated in parallel except that different ssDNA (i.e. complementary ssDNA) were attached on the AFM tip and the glass surface, respectively. The experiment then just consists of approaching the AFM to the surface till a bond (due to hybridization) forms between the complementary ssDNA strands (Figure 1A., upper panel). Note that the use of spacers in this case (i.e. short molecules) is of prime importance because this reduces non-specific interactions. For OT experiments, Hegner (Hegner, 2000) developed a procedure for covalent binding of DNA molecules to polystyrene microspheres. Briefly, it consists (i) to chemically activate microspheres and covalently attach a thiol- or amino-modified 5-end of a dsDNA to the surface of the bead (ii) to attach a ligand (e.g. biotin) to the opposite 3-end. The ligand can then interact with receptors immobilized on a second set of beads, as shown in Figure 1B. (upper panel). This procedure allows preparation of DNA-microspheres in advance and storage of these beads for months. Moreover, high forces can be reached (200 pN) (Hegner *et al.*, 1999; Wuite *et al.*, 2000). This is not possible when using digoxygenin–antidigoxygenin recognition (i.e. non-covalent coupling) (Leger *et al.*, 1998; Merkel, 2001). Notice that a similar procedure was applied in magnetic tweezers experiments (Haber and Wirtz, 2000).

Non-covalent coupling is the most widely used method in OT experiments, certainly due to the ease of preparation. Many groups have worked on lambda DNA or its linearized fragments, which can be labeled at both its 5-ends with biotinylated nucleotides (Figure 1B., lower panel) using nucleotide incorporation with DNA polymerase enzymes. In contrast to covalent coupling, both ends have the same bioreactive group (biotin) and no beads stock solution can be prepared (Davenport *et al.*, 2000).

Finally, DNA can be immobilized between a surface and a tip of an AFM cantilever using non-specific interactions (Rief *et al.*, 1999) (Figure 1A., lower panel).

Overview on single-DNA experiments

Mechanical properties of bare DNA under physiological conditions

Low-force regime of dsDNA

Figure 2 shows a typical dsDNA force vs. fractional extension (x/L) curve obtained with a modified linearized pTYB1 plasmid (7477 bp). For this experiment, a 20-bp thiol-labeled dsDNA linker was ligated to the 5-end of the fragment and the single strand overhang of the 3-end was biotinylated with DNA polymerase

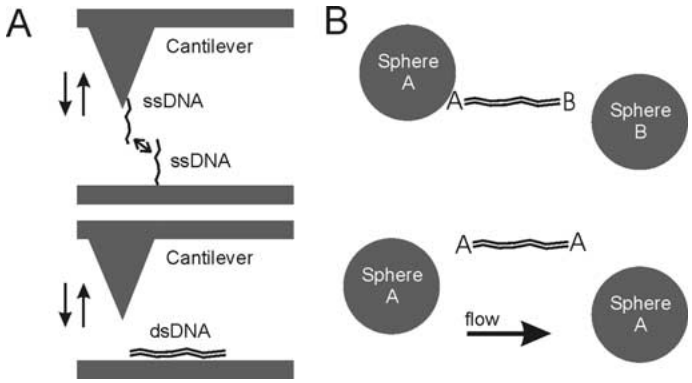

Fig. 1. Possible experimental arrangements for stretching single dsDNA molecules using either a SFM (A) or OT (B) in liquid and room temperature. A. Top panel: complementary ssDNA molecules are covalently attached to a treated AFM tip and a treated glass (Strunz *et al.*, 1999) or gold surface (Boland and Ratner, 1995). When approaching the tip to the surface, a bond may form between the two complementary ssDNAs (DNA hybridization). Bottom panel: an untreated tip is pressed onto an untreated glass surface. Due to non-specific interactions, dsDNA can be picked up and subsequently stretched (the tip has to be pressed to the surface and large forces (up to a few nN) have to be applied). B. Top panel: a dsDNA molecule is covalently attached to a chemically modified polystyrene microsphere (bond A), which is held by the optical trap. The free end of the dsDNA molecule has been labeled with a ligand that can interact with receptors immobilized on a sphere (held by suction on a moveable micropipette) (bond B). This bond (B) can be a weak non-covalent bond (e.g. biotin–streptavidin) (Hegner, 2000). Bottom panel: two microspheres are coated with identical receptors (e.g. streptavidin). One of the beads is held by the optical trap, while the second is held by suction on a moveable micropipette. A dsDNA, which is modified at its opposite 5′-ends with biotin ligands, is injected in the chamber and a continuous flow is applied. Upon attachment of the DNA to the bead in the optical trap (bond A), the measured drag force on the bead increases. The pipette is then moved in the vicinity of the optical trap to attach the free end of the DNA to the pipette bead (bond A). For this experiment, continuous flow is required to prevent looping (Davenport *et al.*, 2000).

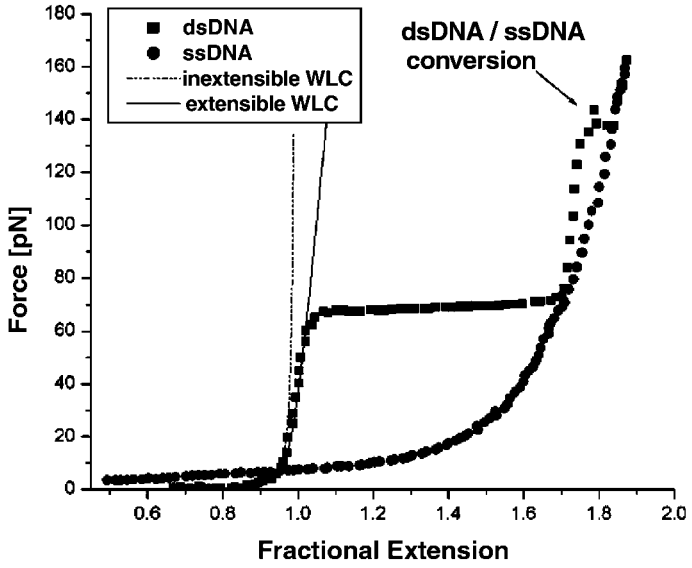

Fig. 2. Conversion of dsDNA into ssDNA (OT experiment, pTYB1 plasmid). For dsDNA single molecules (squares), the force vs. fractional extension (x/L) curve shows a typical overstretching plateau at 68 pN (150 mM NaCl). At low forces (smaller than 5–10 pN, entropic regime), the mechanics of dsDNA can be well described in terms of an inextensible WLC model (Equation 2, dots). At higher forces (smaller than 68 pN), enthalpic contributions have to be taken into account to correctly describe the observed mechanics [extensible WLC model (Equation 3, solid line)]. dsDNA can be converted into ssDNA if a high force (about 140 pN) is applied (circles).

enzymes (Husale *et al.*, 2002). Covalent coupling to polystyrene microspheres was performed in a similar procedure as described in the previous section (Hegner, 2000). At low forces [smaller than 10 pN (Odijk, 1995)], the mechanics of the dsDNA (squares) follows a WLC behavior [Equation (2), Figure 2 (dots)] with a persistence length A of 50 nm at 150 mM NaCl and pH 7.2 (Bustamante *et al.*, 2000a; Strick *et al.*, 2000b). Note that for thick polymer where the sub-units are held together by several bonds in parallel (e.g. dsDNA), the FJC model fails to describe the observed mechanics (Merkel, 2001). Although OT are widely used to investigate stretching of single molecules at low force (entropic regime), we would like to point out that magnetic tweezers is always a technique of choice due to its high force-resolution (see Introduction).

As described previously, an extensible WLC model [Figure 2 (solid line)] provides an excellent agreement

for forces in the range of 10–68 pN. From a linear fit, enthalpic parameters such as the stretch modulus can be determined. Reported values at standard buffer conditions (150 mM NaCl, pH 7.2) are in the order of 1500 pN for bare dsDNA (Smith *et al.*, 1996; Bustamante *et al.*, 2000a).

Overstretching transition of bare dsDNA

At forces of about 68 pN (Cluzel *et al.*, 1996; Smith *et al.*, 1996; Rief *et al.*, 1999), a highly cooperative transition can be seen: over a range of 2–3 pN, the dsDNA is stretched to about 1.7 times its natural contour length (B-DNA form). The occurrence of this plateau, first seen with OT (Smith *et al.*, 1996), is still a subject of debate. Different explanations, either force-induced melting (Rouzina and Bloomfield, 2001a, b) or structural transition (S-DNA) (Lebrun and Lavery, 1996; Haijun *et al.*, 1999) have been proposed to explain the experimental observations. Due to (i) the high cooperativity and (ii) the observed hysteresis while relaxing the molecule, the overstretching plateau could be caused through a force-induced melting (i.e. conversion of dsDNA into ssDNA). When subsequently stretching the dsDNA apart its opposite 5–5 (Smith *et al.*, 1996) or its 5–3 ends (Hegner *et al.*, 1999; Husale *et al.*, 2002), the molecular link should either fall apart (5–5 pulling) or the force vs. extension curve should show a behavior typical of ssDNA (5–3 pulling). However, neither of these phenomena are observed in experiments. Moreover, it was shown that partially cross-linked DNA (5% intercalation of psoralen) only slightly modified the overstretching transition (Smith *et al.*, 1996). For this reason, many groups have suggested that B-DNA undergoes a structural transition into a new form called S-DNA. To date, calculations based on this assumption (i.e. DNA remains in a dsDNA form during the overstretching transition) were not able to predict the experimental trends (e.g. width of the transition, or value of the overstretching plateau). In contrast, models based only on purely thermodynamical arguments found that melting of B-DNA should occur at 60–80 pN of external applied force and correctly estimated the temperature-, salt- and pH-dependence of the overstretching plateau (Williams *et al.*, 2001a, b; Wenner *et al.*, 2002).

Mechanical properties of bare ssDNA under physiological conditions

Also shown in Figure 2 (circles) is the elastic property of bare ssDNA (150 mM NaCl, pH 7.2). Note that ssDNA was directly obtained from dsDNA by applying a high tension (see arrow in Figure 2) on the single molecule (Hegner *et al.*, 1999). For single-chain polymer, it is believed that a FJC model is applicable. This is especially true at 150 mM salt, where ions of different charges are perfectly counterbalanced (theta solvent, no excluded-volume effects). Although the force vs. fractional extension curve can be fitted with a modified FJC model [where the Kuhn segments are allowed to be stretched (Smith *et al.*, 1996)], it is necessary in this case to shrink the expected contour length by ~15%. In contrast, a non-extensible WLC model (with appropriate contour length) qualitatively describes the mechanics up to about 30 pN. Typical values reported for the persistence length are in the order of 0.75 nm (Smith *et al.*, 1996; Rivetti *et al.*, 1998).

AFM vs. *OT and magnetic tweezers*

Although AFM based techniques have been applied in the past to investigate mechanical properties of single polymers, intrinsic relevant parameters such as the persistence length are not accessible to this technique, mainly because of the large thermal noise of commercial AFM cantilever (10 pN, in liquid and at room temperature). However, AFM is a technique of choice to reveal overall mechanical trends, as shown for example by Rief *et al.* (1999). For instance, these studies have carefully studied DNA mechanics in the high-force regime and have shown that (i) the overstretching transition represents thermodynamical equilibrium (independent of pulling rate) and (ii) the melting of dsDNA into ssDNA (observed at higher forces, see Figure 2) is a non-equilibrium process (depends on pulling rate).

Mechanical properties of DNA under non-physiological conditions

When DNA is stretched under non-physiological conditions [change in solvent (Smith *et al.*, 1996), salt (Smith *et al.*, 1992, 1996; Baumann *et al.*, 1997; Wenner *et al.*, 2002), pH (Williams *et al.*, 2001a) or temperature (Williams *et al.*, 2001b)], the mechanics is strongly modified. Varying such external parameters affects both the value of the overstretching force as well as the persistence length. For instance, Smith *et al.* (1992) found an increase of the persistence length A as well as a decrease of the overstretching force when decreasing the salt concentration.

Mechanical properties of coated DNA: binding agents, proteins

We now investigate how mechanical properties are affected when some agents (Ethidium Bromide (EtBr) or SYBR® Green I) intercalate/bind to dsDNA (Figure 3). Although such measurements can be of fundamental interest, such studies can also be of great use for screening purposes without the need of competitive assays (Husale *et al.*, 2002). As expected, an extensible WLC model still provides an excellent description of the mechanical properties when dsDNA is exposed to such binding ligands. For EtBr [1 μg/ml] (Figure 3, stars), we find a decrease in persistence length by factor about two ($A = 25$ nm), a reduction in the stretch modulus of about six ($S = 250$ pN), and a change in contour length of ~25%, respectively. In addition, dsDNA does not show in this case any cooperativity at high force. In contrast (data not shown), SYBR® Green I only slightly

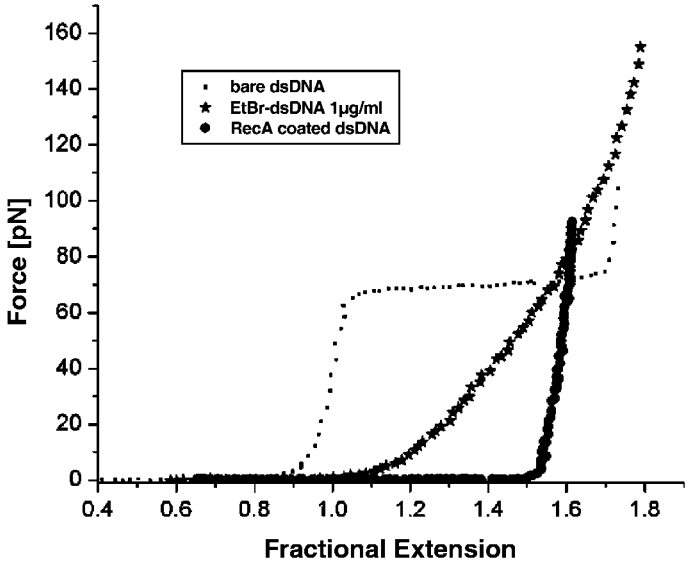

Fig. 3. Comparison of the mechanical properties of (i) naked dsDNA (dots) (ii) dsDNA coated with EtBr (stars) (Husale *et al.*, 2002) (ii) dsDNA coated with RecA (circles) (150 mM NaCl). For this experiment, a pTYB1 dsDNA plasmid (7477 bp) was used. The change in persistence length is well evidenced by the change in slope steepness when approaching the contour length. Coated dsDNA with EtBr (RecA) has a smaller (higher) persistence length than naked dsDNA and therefore the force vs. extension curve shows a less steep (steeper) slope upon approaching the contour length. Note the drastic increase of the contour length of bare dsDNA when coated with RecA.

affects the entropic elastic behavior of bare dsDNA mechanics ($A = 40$ nm). This would explain why – among all fluorescence dyes – SYBR® Green is best suited when enzymatic reactions have to be performed (Schäfer *et al.*, 2000). Note, however, that the mechanics are altered at higher forces since we measure a stretch modulus of about 500 pN. Still, a small overstretching plateau can be seen at ~80 pN.

We emphasize that the differences in mechanics obtained for these two latter compounds can be understood easily by the fact that – unlike EtBr – SYBR® Green I does not intercalate between stacked base pairs. Rather, SYBR® Green I is thought to bind in the minor groove of DNA.

Although most Protein-DNA complexes affect the mechanical properties on a local scale only [DNA-enzymes (Bustamante and Rivetti, 1996; Bustamante *et al.*, 2000b), initiation protein complexes (e.g. tbp)], some of them act on a larger scale (from a few tens of bps to a few thousand). For instance, RecA is known to lengthen and stiffen DNA chains (Figure 3, circles) to facilitate the pairing of homologous sequences (Rocca and Cox, 1997). Hegner *et al.* (1999) investigated the polymerization of RecA on dsDNA and the mechanics of both RecA-dsDNA and – ssDNA complexes. In agreement with biochemical ensemble experiments, the study of mechanical properties of RecA-dsDNA filaments showed that RecA binds to one DNA strand, while the second strand does not adhere tightly to the RecA helix and that this strand is able to slide past the protein component.

Finally, studies have been performed on single chromatin fibers to reveal how assembly and disassembly of chromatin takes place on dsDNA upon pulling (Cuy and Bustamante, 2000; Bennick *et al.*, 2001). At low force (smaller than ~10 pN), nucleosomes are able to assemble and the fiber can be reversibly stretched without disassemble the chromatin structure. Interestingly, the force vs. extension curves show discrete, sudden drops at higher forces. This suggests an irreversible dissociation of the nucleosomal core particles from the DNA.

Twisting dsDNA

Magnetic tweezers have the capability to easily and accurately rotate magnetic handles (see Strick *et al.*, 2000b for a recent review). It makes it therefore possible to study (i) torsionally constrained DNA single molecules [for instance the B to P-DNA transition where the helical pitch is strongly reduced (Allemand *et al.*, 1998)] and (ii) the relaxation of supercoiled DNA by individual enzymes (e.g. topoisomerase) (Strick *et al.*, 2000c).

Unzipping and unbinding dsDNA

In the preceeding sections we have seen that stretching or twisting a single DNA molecule yields important information on either the mechanics (persistence length, stretch modulus) or the stress-induced transitions (overstretching transition, P-DNA form, etc.). Additional information can be obtained when unzipping or unbinding dsDNA molecules, as briefly described below.

Despite inherent experimental and theoretical limitations, unzipping experiments might have potential applications for large-scale DNA sequencing (Essevaz-Roulet *et al.*, 1997; Rief *et al.*, 1999). An interesting phenomenon observed in such experiments was the occurrence of stick-slip events, similar to what can be

found on a macroscopic scale (Bockelmann *et al*., 1997). In other words, strain energy accumulates while stretching the dsDNA single molecule (the force increases) and at a certain force threshold (different for A–T and C–G base pairs) the DNA unzipps in a cooperative manner. These experimental findings have stimulated a number of theoretical investigations (Lubensky and Nelson, 2000; Cocco *et al*., 2001; Bockelmann *et al*., 2002).

Closely related experiments to unzipping are unbinding experiments, where the force needed to fully separate both strands is recorded. Unbinding forces of weak, non-covalent bonds have been measured by SFM (Dammer *et al*., 1996) or biomembrane force probes (Evans and Ritchie, 1997). Initially, these SFM measurements focused on feasibility studies to measure single biomolecular interactions. For instance, Boland and Ratner (1995) have first tried to measure the force necessary to break a single DNA base pair. But recently a few groups showed that these single-molecule experiments give a direct link to ensemble experiments where thermodynamic data are measured. Moreover, such studies give insight into the geometry of the energy landscape of a biomolecular bond (Evans, 1998; Strunz *et al*., 2000; Evans, 2001; Merkel, 2001). An inherent feature of these experiments is that unbinding forces depend on the rate of loading and on the details of the functional relationship between bond lifetime and the applied force. These points have been addressed in details for dsDNA: (i) loading rate and length (bp) dependence (Strunz *et al*., 1999) and (ii) entropic contributions to the energy landscape (Schumakovitch *et al*., 2002). An important result (in agreement with thermodynamic data) was the finding – on the single-molecule level – of cooperative unbinding of base pairs in the DNA duplex.

DNA imaged by SFM

SFM is capable of generating images within ranges of resolution that are of particular interest in biology. Although true atomic resolution may not be possible with biological samples at the moment, a great deal of information can still be obtained from images that show details at a slightly lower level of resolution (Bustamante and Rivetti, 1996; Fotiadis *et al*., 2002). To date, only a few scanning tunneling microscopy (STM) experiments have been reported on DNA (Guckenberger *et al*., 1994; Tanaka *et al*., 1999). Since STM ope-ration in native conditions (i.e. liquid) is difficult to achieve, we believe that SFM is the technique of choice. For this reason, STM experiments will not be discussed in this review.

Imaging techniques

A decade ago, imaging of dsDNA under ambient conditions was performed using contact mode imaging were a cantilever tip is scanned across the surface while applying a constant force. The advantage of SFM over other techniques is that (i) direct imaging is possible without staining of the molecule and (ii) a simple deposition techniques onto flat surfaces can be applied. Erie and colleagues (Erie *et al*., 1994) imaged lambda Cro protein when bound as a single dimer or multiple dimers to its three operator sites on dsDNA. They observed that bending of the non-specific sites is advantageous for a protein such as Cro that bends its specific site, because it increases the binding specificity of the protein. First images of dsDNA in liquids using contact mode showed that technical improvements were needed to allow higher resolution for imaging dsDNA interacting with enzymes (Hegner *et al*., 1993) (Figure 4).

Nowadays, the dynamic-mode operation is the method of choice to image DNA molecules (in air or in liquids). Rivetti *et al*. (1999) analyzed the structure of *E. coli* RNA polymerase-sigma open promoter complex. The high-resolution capabilities of SFM allowed a detailed analysis of a large number of molecules and showed that the dsDNA contour length of open promoter is reduced by approximately 30 nm (90 bp) relative to the free dsDNA. The dsDNA bend angle measured with different methods varied from 55° to 88°. This strongly supported the notion that during transcription initiation, the promoter DNA wraps nearly 300° around the polymerase.

Mechanics of DNA deposited on flat surfaces

An important point to be considered is the fact that for imaging, the DNA is required to be deposited on a flat surface and that this deposition can affect parameters involving DNA mechanics. If proteins have to interact with the DNA, the conditions for binding the DNA to a surface might not be suitable for an optimal protein-DNA interaction.

Rivetti *et al*. (1996, 1998) developed suitable procedures to deposit DNA molecules onto freshly cleaved mica. This method allows the DNA to equilibrate on the surface as in an ideal two-dimensional solution. Under equilibration conditions, this study indicated that the SFM can be used to determine the persistence length of DNA molecules to a high degree of precision. They reexamined the DNA length measurement recently and revealed a discrete, size-dependent, shortening of DNA molecules deposited onto mica under low salt conditions (Rivetti and Codeluppi, 2001). Awareness of this structural alteration, which can be attributed to a partial transition from B- to A-form DNA, may lead to a more correct interpretation of DNA molecules or protein-DNA complexes imaged by SFM in the future.

We have shown previously that small agents can considerably affect the mechanics. In a recent study, Coury *et al*. (1996) presented a procedure to detect these properties by measuring the contour length of the

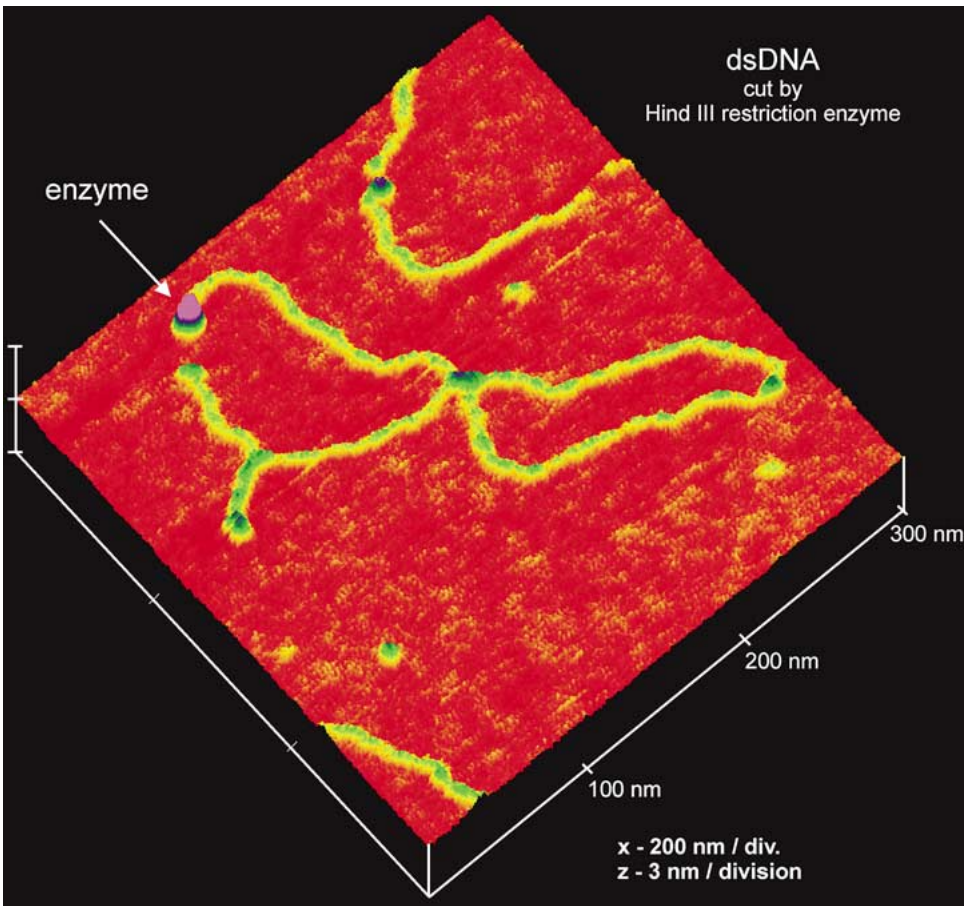

Fig. 4. Tapping-mode SFM image of an enzyme (HindIII) interacting with a plasmid DNA (pNEB193). As suggested by the 5 nm gap visible next to the enzyme, the deposition of the protein DNA complex took place immediately after the digestion of the closed plasmid. Measurements were made under ambient conditions [deposition buffer 4 mM Hepes pH 7.2, 10 mM NaCl, 2 mM $MgCl_2$ (Rivetti *et al.*, 1996)].

dsDNA molecule using SFM. They incubated the bare DNA molecules with the specific agent and subsequently deposited the modified molecules onto a mica surface. They investigated the amount of extension relative to the contour length. It was shown that the fraction of bound molecules could be estimated and an affinity could be determined by subjecting the DNA to various amounts of ligands. Such an approach reveals some of these parameters, but has the drawback that molecules have to be placed onto a surface in order to be accessible to the SFM, which affects the binding of the ligand to the DNA.

Protein-dsDNA interaction measured in physiological buffer solutions

The group of Bustamante provided a series of images with macromolecular resolution, which showed details on the mechanisms by which RNA polymerase non-specifically translocates along DNA (Guthold *et al.*, 1999). The dynamics of nonspecific and specific *Escherichia coli* RNA polymerase (RNAP)-DNA complexes were directly observed using SFM operating in buffer solution. Imaging conditions were found in which DNA molecules were adsorbed onto mica strongly enough to be imaged. Moreover, the same molecule is bound loosely enough to be able to diffuse on the surface. In sequential images of non-specific complexes, RNAP was seen to slide along DNA, performing a one-dimensional random walk. Note that in these studies the enzyme remained fixed on the surface whereas the DNA was sliding through (along) the polymerase enzyme. It was observed that mica-bound transcription complexes showed a transcription rate, which was about three times smaller than the rate of complexes in solution. This assay confirmed that the biological function of the enzyme is considerably affected by the surface but that 'native' imaging is possible using SFM in liquids.

Mapping of dsDNA with SFM and time-lapse imaging of protein-DNA interactions

Some other specific highlights in the literature include the mapping of DNA using restriction enzymes (Allison *et al.*, 1996, 1997). These experiments showed that SFM can be used to perform restriction mapping on individual cosmid clones. A mutant EcoRI endonuclease was site-specifically bound to DNA. Distances between endonuclease molecules bound to lambda DNA were measured and compared to known values. These

experiments demonstrate the accuracy of SFM mapping to better than 1 kbp. These results may be extended to identify other important site-specific protein-DNA interactions, such as transcription factor and mismatch repair enzyme binding, which are difficult to resolve by other techniques. In addition, time-lapsed microscopy was used to observe dynamic interactions of proteins on dsDNA. In these series of experiments, dynamic interactions of the tumor suppressor protein p53 with a DNA fragment containing a p53-specific recognition sequence were directly observed by time-lapse tapping-mode SFM in liquid (Jiao *et al*., 2001). As in other studies (Guthold *et al*., 1999) the divalent cation Mg^{2+} was used to loosely attach both DNA and p53 to a mica surface so that they could be imaged by the SFM while interacting with each other. Various interactions of p53 with DNA were observed, including dissociation/reassociation, sliding and possibly direct binding to the specific sequence.

These authors visualized two modes of target recognition of p53: (a) direct binding, and (b) initial non-specific binding with subsequent translocation by one-dimensional diffusion of the protein along the DNA to the specific site. It is to be foreseen that in the future, i.e. when both higher resolution (Li *et al*., 1999) and faster imaging in liquids (time to require one image <1 min) will be routine, time lapsed-imaging of SFM in liquids will allow to get insights of unprecedented quality and dynamics.

Acknowledgements

Financial support of the NCCR 'Nanoscale Science' the Swiss National Science Foundation and the ELTEM Regio Project Nanotechnology is gratefully acknowledged.

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