Stretching, Tearing, and Dissecting Single Molecules of DNA**

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Chemists usually sample Avogadro numbers of molecules for their experiments. The properties they measure are averaged on the entire ensemble of those molecules and over all conformations accessible to them. A new exciting dimension has now been opened: A single molecule can be simultaneously touched and observed, and then also dissected and mechanically manipulated to drive its structure towards new conformations and states that otherwise can be very hard to reach.

Bulk studies have shown that DNA can undergo transitions from the native B form to the A and Z forms, but the repertoire of structural changes open to the double helix is much wider than that. For instance, during recombination the RecA protein stretches DNA beyond its natural contour length. DNA strands are locally unzipped and pulled apart by RNA polymerase during transcription. Topoisomerases underwind DNA, thus forcing its chain to supercoil around itself like a telephone cord does when we twist the receiver. A remarkable series of experiments, carried out in the last few years in several laboratories, was able to reproduce and study all these DNA structural modifications in single molecules. These investigations started from the pioneering work of Bustamante and co-workers, who made force versus extension measurements on a single DNA molecule that had been anchored on one side to a glass slide and on the other to a magnetic bead.[1] They determined that forces of 5 pN were required to stretch an individual λ-DNA (bacteriophage) dimer (97 kb) to nearly its full contour length. This experiment provided the strictest test of entropic polymer elasticity to date, and it also demonstrated the limitation of the freely jointed chain (FJC) model with respect to the wormlike chain (WLC) model for a polymer such as DNA. While the former model represents the DNA as a chain of rigid rods connected by revolving pivots, the latter treats DNA as a uniform elastic rod and describes its path as a continuum curve.[2]

Optical tweezers (OT) and bendable microneedles (BM), obtained by pulling optical fibers under heat to microscopic dimensions, were introduced as force sensors in subsequent experiments where the DNA could be pulled beyond 10 pN. Unlike the low force or entropic elasticity regime, this intermediate force or "intrinsic elasticity" regime has an enthalpic origin, which corresponds to the stretching of the molecule through the straining of the chemical bonds and the gradual deformation of the double helix. The research groups of Bustamante and Caron independently found that by applying forces in the range of 20–50 pN they could stretch the DNA beyond its natural length and, surprisingly, when they increased the force to about 70 pN the DNA yielded abruptly and extended to almost twice its contour length: It underwent a sharp and reversible transition to a new distinct form of extended DNA.[3, 4]

At the end of their paper on the entropic elasticity of DNA,[1] Smith et al. suggested the possibility of introducing the capability to simultaneously twist and pull a DNA molecule by rotating the magnetic field in their magnetic-bead manipulation tool. The twisting of the bead over- or underwinds the molecule, thus forcing it to supercoil. This experiment, carried out a few years later by Strick et al.,[5] showed that a little tension (about 5 pN) can prevent the writhing of the chain by converting the deficit in the linking number into a deficit in the number of twists in the Watson–Crick helix. This process originates melted regions in the helix and is a very important result for our understanding of transcription. RNA polymerase (RNAP) overwinds DNA ahead and underwinds it behind itself during its tracking of the double helix.[6] Guptasarma proposed recently that the underwound DNA behind a RNAP assembly can melt and form new transcription bubbles.[7] The experiment by Strick et al.,[5] if considered together with the recent demonstration by Yin et al.[8] that RNAP can exert a force as large as 14 pN on the DNA molecule, shows how these bubbles could be produced.

The most recent force versus extension measurements in the two regimes of intrinsic elasticity and overstretching of the DNA were carried out by Shivashankar and Libchaber with a scanning force microscopy (SFM) apparatus.[9] One end of a linear λ-DNA molecule was grafted onto a glass coverslip with a biotin–streptavidin linker and the other end to a 3.2-μm latex bead, again with the same linker. This DNA-tethered bead was brought into contact and attached to a SFM cantilever by means of an optical tweezer. The DNA molecule was extended by moving the bead away from the coverslip. The forces, measured from the deflection of the cantilever, were basically the same as those obtained by the other groups with optical tools.[3, 4] This coincidence could raise doubts about the reliability of those measurements, because, as we will see in a while, different manipulation tools are expected to drive the experiments along different energy pathways. But the doubts disappear if we analyze these data in terms of force loading rates (see below and reference [17]).

DNA was more recently subjected to a much worse "torture". Essevaz-Roulet et al. opened a single λ-DNA molecule by pulling apart its complementary strands, like the two sides of a zipper.[10] At one end of the double-stranded DNA molecule one strand was linked to the microscope slide and the other strand to the bead. The other end of the molecule was capped with an oligonucleotide forming a

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hairpin (Figure 1 a) to prevent separation of the two strands when reaching the end of the opening process and so allow unzipping–zipping cycles to occur. The mechanical forces that were required to separate the strands were in the range of 10 to 15 pN per base pair. Figure 1 b reports the fluctuations in the force required to directionally melt the double helix and

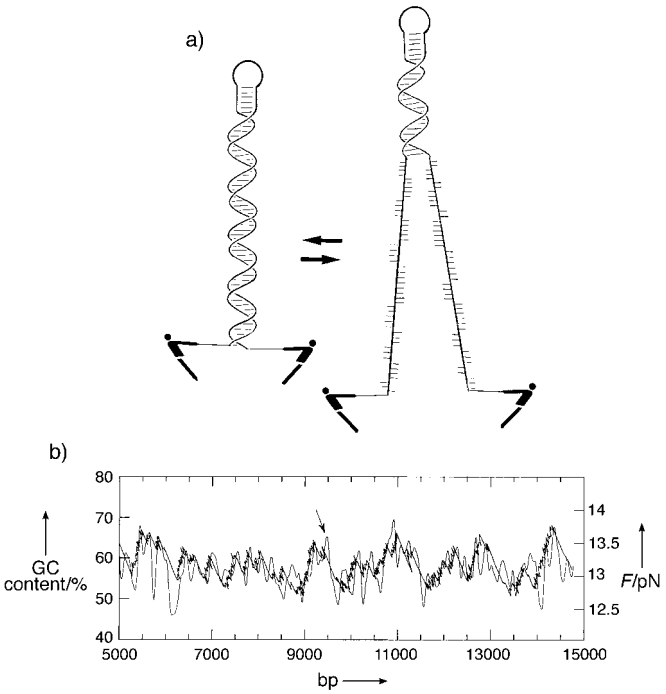

Figure 1. a) Cartoon representation of the unzipping experiment carried out by Essevaz-Roulet et al.[10] on a single λ-DNA molecule (48.5 kb). b) Comparison between the average GC content [%] along a segment from 5000 to 15000 bp of the sequence of λ-DNA (smooth curve) and the fluctuations of the force F required to directionally melt the same segment (less smooth curve). The GC content was averaged over 100 bases. (Reproduced with permission from reference [10], copyright (1997) National Academy of Sciences, U.S.A.)

shows that the forces are related to the GC and AT content of the DNA sequence. The peaks in the graph correspond to a higher GC content, which transiently blocks the opening of the double helix until the force reaches a level where the opening occurs quickly in the chain segment that follows. In the complete opening of the DNA sequence, the number of these events was one every 500 bases. This kind of resolution is probably still too low for these mechanical measurements to become a source of intermediate-scale information on long pieces of DNA that could complement and support enzymatic sequencing methods, as suggested by the authors.

Much higher sequence resolution was achieved in a similar experiment carried out by Lee et al. with an SFM apparatus.[11] They covalently linked one end of a single-stranded oligonucleotide that was 20 base-long to a spherical probe attached to a SFM cantilever. The probe was brought into contact with a surface where the complementary single-stranded oligonucleotide had been tethered, so that annealing could be attained. By pulling away the probe they sheared the 20 base strands at once: The forces they used were of the order of 1000 pN, about 50 pN per base pair. This is a much larger value than that measured by Essevaz-Roulet for breaking base pairs.[10] The difference in values could be the result of different geometries of pulling, but also other explanations can be proposed. As once suggested to me by Steve Smith, in Lee's experiment the molecule should go into the extended form at 70 pN[3, 4] and then fray (melt) from its ends. That process may take too long, hence a higher force is required (see below). Alternatively, it may be that the force they measured was that of breaking double-stranded DNA instead. Bensimon et al. estimated this force to be about 470 pN.[12]

The rupture forces between base pairs were measured in another SFM experiment by Boland et al.[13] Self-assembled monolayers of purines and pyrimidines were deposited onto gold-coated surfaces and onto gold-coated SFM tips. The directional hydrogen-bonding interaction was measured between complementary and noncomplementary individual bases. A separation force of 54 pN was reported for a single AT base pair: This value obtained by SFM was much larger than that found with BM.[10] One further reason for these larger values can be envisaged. The SFM methods were developed to map surfaces with a very high vertical resolution. Their precision in measuring the extension in manipulation experiments is, therefore, very high and makes it possible to investigate much shorter DNA molecules than with OT or BM. This is a great advantage but, at the same time, the working distance could become so small that nonspecific interactions between the tip and the surface holding the other end of the molecule could be included. These interactions can contribute to increase the force in the SFM experiments.

The general approach of all the experiments described above is to induce ruptures of noncovalent interactions by applying an external force to lower the activation barrier as much as necessary to trigger, on the time scale of the experiment, an otherwise unlikely event.[14–16] In these experiments the surmounting of the potential barrier is expected to be still thermally activated. This can take place provided that: 1) the applied force is not large enough to eliminate the activation barrier and 2) the pulling velocity is slow enough to leave the molecule thermally fluctuating at the barrier for a time compatible with a thermally activated process. The width of the thermal fluctuations within the potential well is determined by the "stiffness" of the tool which loads the external force. The force loading rate, as defined by Evans and Ritchies,[14] is thus the most useful dynamic parameter that can be utilized in the analysis of force data.[17]

These force-directed processes are accompanied by irreversible work (viscous friction force), which is spent in addition to the work required by the thermodynamic potentials. As shown by Schultzen and colleagues, the thermodynamic potentials of the processes under investigation cannot be reconstructed because of these irreversible components of the work and because of the superposition of an external potential.[15] This is a limitation of this type of experiments if we are interested in equilibrium processes. On the other hand they open very attractive prospects of tailoring the manipulations of single molecules for studying nonequilibrium

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reactions. Features of nonequilibrium "energy landscapes"[15] can be explored along the different pathways we can select by changing the stiffness of the tool and the pulling rate.

The SFM method offers one further opportunity for micromanipulating single molecules. Its probe, after having imaged a single molecule, can be stopped at a given position and used there as a dissecting scalpel. The first experiments of this type were carried out in air by James Vesenka et al.[18] and Eric Henderson.[19]

Figure 2 shows a circular DNA molecule transformed into a linear molecule in solution by a double-strand cut that is induced by the local pressure of the SFM tip. This experiment

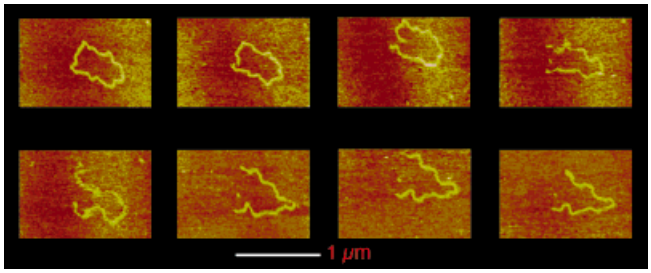

Figure 2. SFM images (tapping mode, deionized water) of pBR322 plasmids deposited on mica from a buffer solution (4 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), 1 mM $MgCl_2$, pH 7.4). The relaxed plasmid displayed in the first picture was cut in situ, while imaging, by the SFM tip. The linearized molecule started searching amongst conformations with a higher end-to-end distance than its initial zero value. In the fourth frame the molecule already bore very little resemblance to its original shape. We can modulate the adhesion of the molecule on the surface of mica by changing the ionic strength of the solution.[20] An injection of water, after the sixth frame, improved the resolution owing to the reduction in the molecular mobility. This experiment was carried out by G. Zuccheri.[21]

was performed in our laboratory, and a linear DNA molecule was thus created in situ. Also this single molecule manipulation creates a structure which is expected to be very poorly populated: The distance between the two newly created ends of the linear molecule was almost zero. As this experiment was carried out in liquid, we could switch the SFM back to the imaging mode after the cut, and see the two ends move apart. We could observe, in real time, the conformational rearrangement that led this molecule to an end-to-end distance of the order of magnitude theoretically expected to be the most populated for a polymer of its length.

Topics of polymer chemistry and biology can now be transferred to the single-molecule level. Our understanding of many issues has been limited so far because the traditional bulk measurements are averaged over unknown molecular distributions and not necessarily homogeneous. Experiments carried out on individual molecules can cast a new light on these issues. Further advances in the theory are still necessary for both the tailoring of the experiments and the interpretation of the data. The methodologies are being developed very rapidly, and future advances will depend on technical improvements in the time resolution of the measurements and the mechanical and thermal stability of the manipulation tools. The measurement of the spring constants is still very cumbersome and poorly reliable. A Dutch proverb says that "everything has its science, with the exception of catching fleas: that is an art". Also "catching" individual molecules is still quite an art, but no fundamental limitations appear to prevent it from becoming an established tool offered to the ingenuity of chemists and biologists.

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