New and Notable

Twisting DNA Molecules

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Understanding the biological mechanics of DNA is largely a matter of understanding the molecule's response to torsion. Excess twist is stored in supercoils that may be restrained by wrapping around histone cores or unrestrained as required in replication and transcription. DNA's torsional state is so critical that different topoisomerases have evolved to introduce twist or to relieve it (Drlica, 1992), yet little is known about the way in which torsion mediates the interactions between DNA and essential proteins such as histones or RNA polymerase. Regions of a prokaryotic or eukaryotic chromosome that display unrestrained supercoiling are especially interesting because they correspond to actively transcribed genes (Schmid, 1988; Kramer and Sinden, 1997). How elastic are these genomic regions? How hard must something (e.g., a molecular motor protein) pull to stretch a supercoiled loop or to translate a coiled gene region past an immobilized RNA polymerase complex?

In their article in this issue, "Behavior of Supercoiled DNA" T. R. Strick, J.-F. Allemand, D. Bensimon, and V. Croquette use single molecule manipulation to answer some of these questions and to shed new light on the well-known phenomenon of DNA supercoiling. These authors attached individual DNA molecules to magnetic beads and measured the end-to-end extension of the molecules versus applied tension. Then, by rotating the beads, they added various amounts of twist to the DNA molecules and caused them to supercoil, as evidenced by changes in their force versus extension curves (see their Fig. 5). Whereas relaxed DNA exhibits only entropic ("rubber") elasticity, twisted DNA displays additional enthalpic elasticity due to supercoil formation. The theory for such elasticity is complex because of a partitioning of the excess linking number into various amounts of twist versus solenoidal or plectonemic writhe. This partitioning depends, in turn, on the molecular tension according to the energy of the states and their abundance (entropy).

For small changes in twist ($<1\%$), the results of these experiments agree fairly well with the theory for a twisted elastic rod in a thermal bath (Marko and Siggia, 1995). Indeed, supercoil formation significantly increases the contractile force on the ends of a molecule because it "reels in" those ends. The limits of linear elastic behavior are made obvious, however, because direct manipulation can produce forces on a molecule that exceed normal biological or thermal stresses. Among the surprising findings of this study are the alternative DNA forms that can be induced by changing the molecule's twist. By unwinding DNA, a cooperative transition to a melted state (or possible left-handed helix) can be produced. With overwinding, a different cooperative transition to a new hyperwound state is observed. How can we know these transitions are cooperative? Because they occur over a narrow force (ergo torque) range. Therefore $\Delta G \gg kT$ for the independent changeable unit in a two-state Ising model. But ΔG equals $\sim 1kT$ for a single base pair, and therefore many base pairs must be changing together as a unit. So how do we know ΔG for a single base pair? By the use of single-molecule thermodynamics, as nicely demonstrated in the present article. By integrating force times distance (Δx) during a reversible process, a free energy change is obtained.

The term "single-molecule thermodynamics" might appear as an oxymoron to some readers, because thermodynamics normally deals with an ensemble of separate molecules. DNA, however, is so long that it can be treated as a string of independently thermalized subunits. Furthermore, by observing a single molecule at many different times, a thermodynamic ensemble is produced (ergodic hypothesis). By averaging the forces and extensions for a sufficiently long time, equilibrium averages are obtained. A test for sufficient averaging time is whether the force versus extension curve is reversible, i.e., whether the same force curve is obtained when the molecule is stretched or relaxed. Many single-molecule force curves display hysteresis, e.g., the domain unfolding/refolding force curves for the giant polypeptide titin (Kellermayer et al., 1997). Hysteretic curves are useful for estimating activation energies but useless for obtaining free energy changes. The work of Strick et al. beautifully illustrates the way in which brownian motion and coulombic repulsion "lubricate" the molecular machinery as writhe is converted to twist and vice versa. No energy is lost to friction because the molecule seldom rubs against itself; the supercoils are "inflated" by thermal motion and excluded volume effects; the force curves are reversible and $\Delta G = F\Delta x$. This wonderful machinery breaks down under increased pressure. By strongly overwinding a DNA molecule in high salt, a regime is entered into in which parts of the molecule interact by "rubbing," as evidenced by hysteresis in the force curves (their figure 7).

The new techniques and results reported by Strick et al. open up possibilities for new experiments with even greater biological significance. Virtually any soluable protein can be introduced into the buffer flowing past a tethered molecule. Most of the significant structural or enzymatic proteins that bind to DNA are sensitive to supercoiling; indeed, most require it for proper function. If such proteins bind to and restrain (or remove) supecoils, then that binding should be observable

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as a change in molecular contour length. Consider the case of histones binding to DNA to form nucleosomes. It has been difficult to reconstitute nucleosomes on relaxed DNA in free solution, but that might be overcome by introducing negative twist inside a tethered molecule. If nucleosomes can be reversibly formed or displaced by changing the molecular tension, then the free energy of binding (and the kinetics of binding) can be obtained for different values of the molecular twist.

The beauty of bare DNA experiments lies in the precise and repeatable data obtainable from a single macromolecule (see, e.g., their figure 5). One difficulty for the experimenter, however, lies in the diversity of behavior exhibited by different "single" molecules that are not really identical. Any DNA molecule may be nicked or unnicked, attached to surfaces at points other than its ends, or attached at the pole or equator of the magnetic bead. There may also be multiple molecules attached to one bead. Strick et al. must have tested hundreds of candidate molecules to recognize these different behaviors and select particular molecules that exemplify the simplest states. To such complexity for bare DNA, add the additional number of ways that multiple protein molecules (e.g., histones) could bind to a single molecule, and you may produce a formidable array of behaviors. But isn't real biology always complex?

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