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Commentary

The rise of single-molecule DNA biochemistry

Alexander Rich

Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Room 68-233, Cambridge, MA 02139

We have known about conformational changes in DNA far longer than we have known its three-dimensional structure. Early experiments showing the irreversibility of acid-base titration of DNA and of changes in the ultraviolet absorption associated with heating suggested that changes in conformation were occurring. Experiments with DNA fibers that facilitated the derivation of the double helix by Watson and Crick started with the observation of a conformational change, visualized in fiber x-ray diffraction patterns. The first fibers drawn by M. F. Wilkins, R. Franklin, and their collaborators were usually allowed to dry in air. The air-dried fibers produced a characteristic helical diffraction pattern, which was called the A type. On the other hand, drawing a fiber and maintaining it in the hydrated-state produced a different diffraction pattern, which was called the B type. We speak of B-DNA as the usual form of the double helix in biological systems. When the three-dimensional structures of both the A and B forms were worked out, it turned out that the differences between them are related to a change in the extension of the sugar-phosphate backbone. Thus, the backbone of DNA can be regarded as elastic. The basis of this elasticity is associated with changes in the conformation of the ribose ring. In the A form, the ring has a C3′ *endo* conformation, which has a phosphate-to-phosphate distance close to 5.9 Å. However, in the B form, the ring is in the C2′ *endo* pucker in which there is ≈7 Å between adjacent phosphate groups. The shorter distance in the A form resulted in a wider double helix in which there is actually a hole running down the axis of the helix, with the double-stranded molecule wrapped around it. In the B form, the hole disappears, and the bases form an axial stack in the center of the molecule. Although DNA is predominantly in the B form, it is the A conformation, which is found in double-stranded RNA. The reason that form is stable in RNA is due to the fact that the oxygen atom on the 2′ position of the ribose ring stabilizes the C3′ *endo* conformation, which is generally found in an RNA chain. However, in DNA, the energy barrier between the two different types of pucker is small, and this leads to great flexibility in the DNA duplex. This flexibility facilitates the large number of conformations that the DNA molecule can adopt.

By now, we are quite familiar with the manner in which the DNA duplex can be bent, sometimes as a consequence of sequence periodicities in the molecule and other times when it is associated with specific proteins. An example of significant conformational change in DNA associated with a protein is the complex which forms between DNA and the TATA box-binding protein, where the DNA is untwisted and sharply bent (1). Under the stimulus of torsional stress induced by negative supercoiling, segments of DNA, especially those with alternating purines and pyrimidines, can form another stable structure, the left-handed Z-DNA conformation (2), which was among the first alternative conformations described following the initial discovery of the A and B conformations.

Study of DNA conformational changes often provides significant insight into how the molecule functions. Accordingly, it is of considerable interest that new experimental techniques involving the study of single DNA molecules have been adopted to study conformational changes in DNA. A significant addition to this literature is found in the paper by Allemand *et al.* (3) in this issue of the *Proceedings*, where they show that stretching and overwinding single DNA molecules results in a striking conformational change.

Single-molecule DNA biochemistry is a rapidly growing extension of traditional biochemical techniques. There are many examples to be cited. For instance, molecules of DNA can be oriented and attached to a flat surface. Treatment with restriction endonucleases can introduce gaps in the oriented DNA. Application of a fluorescent dye makes it possible to measure the remaining segments by their fluorescence. In this way, a DNA restriction map can be deduced by an automated system using optical mapping of chromosome segments (4).

An increasingly important type of research on DNA is carried out by anchoring the end of the DNA strand to a small magnetic bead (5) controlled by magnetic forces or to a polystyrene bead 0.5 μm in diameter. The polystyrene bead is held in a low-energy spot at the center of a laser beam. The "optical tweezers" can be used to measure how much force is exerted on the DNA molecule. In one application, RNA polymerase is attached to a surface, and the pull generated by that enzyme on the DNA can be measured (6). This experiment shows that it is an effective motor enzyme since it generates 14 pN of force. Other motor proteins are known to generate pulls up to 6 pN. The power associated with the movement of the polymerase is not entirely surprising in view of the large number of obstacles on the DNA strand, including nucleosomes and proteins which must be disassembled before transcription can proceed. Movement of the polymerase also is associated with forming relatively high levels of negatively supercoiled DNA behind it and positive supercoiling in front (7).

When both ends of the DNA molecule are attached, one end to a bead and the other end to a flat surface, many properties are revealed. Single molecules of double-stranded DNA have been stretched by using the laser tweezer, which can measure the amount of force applied to it. Near the stretching force level of 65–70 pN, double-stranded DNA molecules undergo a cooperative transition in which there is a 5.8 Å rise per base pair, and the DNA is 70% longer (8, 9). This transition is reversible. There is good reason to believe that *in vivo* DNA may at times be subjected to considerable extension force. For example, rec A mediated recombination involves stretching DNA to 1.5 times its B-form length. A model made to account for the transition suggests that the bases have rotated so they are now oriented along the axis, rather than perpendicular to it. Early experiments in which DNA fibers were pulled were often associated with an unusual transition. Pulling the fiber too rapidly resulted in a necking down of the fiber and a change from negative birefringence, which is found initially in the fiber to positive birefringence in the necked-down area. It is likely that the necking behavior is associated with reorientation of

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the bases so that they are more parallel to the helix axis than perpendicular to it.

In other experiments, negatively supercoiled single molecules were stretched, and this activates homologous pairing as indicated by the hybridization of small DNA fragments (10). This result occurs only with small stretching forces in the range of 2 pN, and it has led to the suggestion that this type of stretching may facilitate the opening of the DNA duplex that is a prelude to homologous recombination. Typically, the denaturation bubbles that are formed in the process occur in regions rich in AT base pairs.

The most recent addition to the field of single-molecule DNA biochemistry is the work of Allemand *et al.* (3). In their experiments, the ends of a 17-kb DNA are attached to either digoxigenin or biotin. A glass surface coated with anti-digoxigenin antibodies holds one end, while a streptavidin-coated magnetic bead secures the other end (Fig. 1). The molecule can be stretched or twisted. When the molecule is slightly twisted, it can writhe, so that it looks like a tangled telephone cord. Pulling on the molecule reduces the writhe but increases the twist. This may induce conformational transitions, which are detected by large changes in extension associated with small increases in force. Instead of changes associated with large forces in the 70 pN range, Allemand *et al.* explored the force region near 3 pN or less, with changes confined largely to supercoiling. They find that twisting a DNA molecule, which is unable to writhe leads to a structural transition, thereby relieving its torque. When the molecule is slightly overtwisted or positively supercoiled, even a modest 3 pN force brings about a new conformation. The structure that is formed is quite different from the one induced by a 70 pN force (8, 9).

Analysis of the relative extension as a function of force leads to the conclusion that the new phase has ≈2.6 base pairs per turn. Molecular modeling suggests that stretching with this small number of base pairs per turn requires that the Watson–Crick base pairs would break, and the bases would be expelled from the interior of the double helix. When this occurs, it allows the backbones to move toward the center of the molecule with only a moderate energy cost. In this example of "inside-out" DNA, the sugar phosphate backbones are at the center of the molecule, and the bases are on the outside. This conformation appears compatible with both a C2′ *endo* and C3′ *endo* pucker; although with higher twists, the molecule would be forced to adopt the C2′ *endo* conformation with the corresponding longer phosphate–phosphate distance. In carrying out this transition, the major changes involve rotation of dihedral angles toward trans conformations to get the major extension. Allemand *et al.* have tested the model by reacting the DNA in this form with glyoxal, which does not react with Watson–Crick paired bases but reacts readily with individual bases. The results support their interpretation.

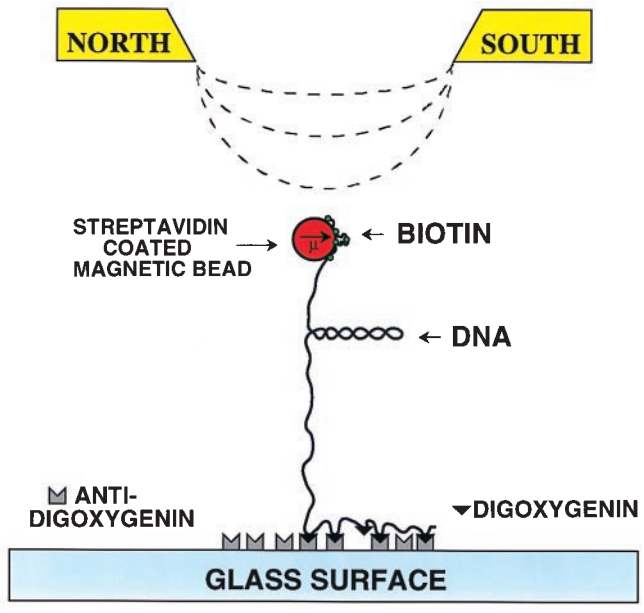

FIG. 1. A streptavidin-coated magnetic bead attached to DNA can be rotated or moved vertically in the magnetic field. This can stretch or twist the DNA attached to a glass slide (adapted from ref. 3).

It is interesting that this transition occurs with only moderate positive supercoiling and in response to quite moderate forces, slightly greater than 3 pN. It is known that RNA polymerase generates positive supercoiling associated with transcription; consequently, it would not be surprising to find this type of conformational change occurring *in vivo* under physiological conditions.

It is possible that protein ensembles also can stabilize this conformation. In the Pf1 bacteriophage the single-stranded circular DNA is stabilized by the helical coat protein (11). From structural studies, it is inferred that two strands of the DNA are intertwined. The structure appears to be formed by twisting two sugar phosphate backbones around each other, so that phosphates are 2.5 Å from the axis in a conformation, which has the bases outside. Allemand *et al.* (3) have used single-molecule DNA biochemistry to uncover a conformation that appears to mirror the conformation found in the DNA packed inside a virus.

It is unlikely that we have heard the last word about DNA conformational changes. There is every prospect that more will be uncovered, and some of the new insights will undoubtedly result from novel studies of single molecules. The field of single-molecule DNA biochemistry is relatively new. As it matures, we will understand how forces at the molecular level of the order of piconewtons underlie the varied chemistries and molecular biology of genetic material.

1. Kim, J. L., Nikolov, D. B. & Burley, J. K. (1993) *Nature (London)* **365,** 520–527.
2. Wang, A. H. J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. & Rich, A. (1979) *Nature (London)* **282,** 680–686.
3. Allemand, J. F., Bensimon, D., Lavery, R. & Croquette, V. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 14152–14157.
4. Cai, W., Jing, J., Irvin, B., Ohler, L., Rose, E., Shizuya, H., Kim, U.-J., Simon, M., Anantharaman, T., Mishra, B., *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3390–3395.
5. Smith, S. B., Finzi, L. & Bustamante, C. (1992) *Science* **258,** 1122–1126.
6. Yin, H., Wang, M. D., Svoboda, K., Landick, R., Block, S. M. & Gelles, J. (1995) *Science* **270,** 1653–1656.
7. Liu, L. F. & Wang, J. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7024–7027.
8. Cluzel, P., Lebrun, A., Heller, C., Lavery, R., Viovy, J.-L., Chatenay, D. & Caron, F. (1996) *Science* **271,** 792–794.
9. Smith, S. B., Cui, Y. & Bustamante, C. (1996) *Science* **271,** 795–799.
10. Strick, T. R., Croquette, V. & Bensimon, D. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 10579–10583.
11. Liu, D. J. & Day, L. A. (1994) *Science* **265,** 671–674.