Upsetting the Balance of Forces in DNA

Donald M. Crothers

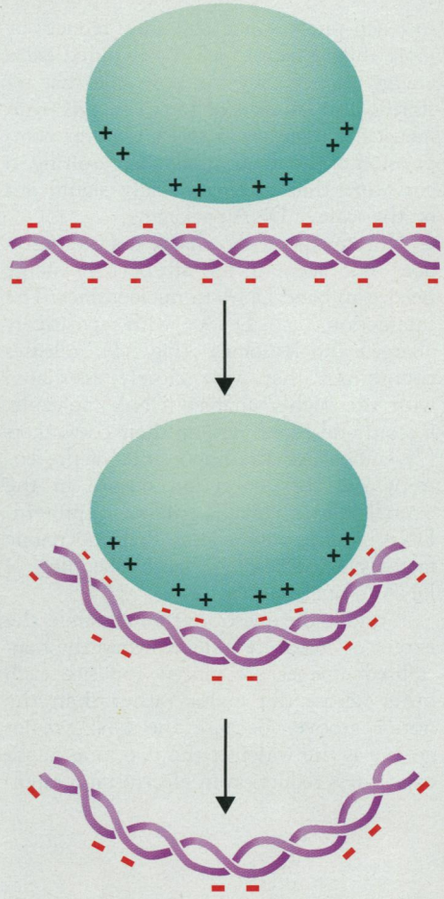

Fig. 1. Bent DNA. DNA approaches a positively charged curved surface. Interaction and formation of salt bridges require bending of the DNA. Neutralization of the charges in salt bridges leaves a DNA helix with an asymmetric charge distribution. Such a structure is intrinsically bent.

The negative charges carried by the DNA phosphodiester backbone follow a double helical path. In a long molecule, this arrangement provides a symmetric charge distribution in which there is no excess electrostatic repulsion among the charges arrayed along any particular face of the helix. What would happen to the structure of DNA if the charges on one side of the helix were selectively neutralized? This question, posed earlier by Mirzabekov and Rich (*1*) and analyzed theoretically by Manning and co-workers (*2*), has now been given an experimental answer by Strauss and Maher on page 1829 of this issue.

The origin of the Mirzabekov and Rich conjecture that DNA should bend as a consequence of selective charge neutralization was the observation of partial cancellation of DNA charge by interaction with lysine and arginine residues when the double helix is bent around core histones to form nucleosomes. As shown in Fig. 1, the initially straight DNA accommodates to the curved surface of the histone octamer by bending so that salt bridges can be formed with the basic residues. The salt bridges result in charge neutralization, leaving, as shown in the last panel of Fig. 1, a bent DNA with an asymmetric charge distribution, grinning back like the Cheshire cat. According to the model, repulsive electrostatic forces from the excess of charge on one face of the helix act spontaneously to create and maintain compression of the opposite helix face and hence bending of the double helix.

Strauss and Maher have attacked this problem by constructing DNA molecules in which selected phosphates are replaced by neutral methylphosphonate analogs. Placing three such residues on each strand so that they are positioned as immediate neighbors across the minor groove, interrupts the repulsive interactions along the corresponding side of the double helix. According to the model, such molecules should be bent. DNA molecules that are bent or curved are anomalous in electrophoretic mobility (*3*); curved molecules run more slowly than straight ones. Strauss and Maher exploited the technique of comparative electrophoresis (*4*) to determine the direction and magnitude of the DNA bend that results from selective charge neutralization.

The key to this approach is to interdigitate segments containing neutralized charges between special DNA sequences that cause a bend of known direction and magnitude. Ligation of monomers containing the two types of bends into multimers provides many repeats of the motif of alternating bend types. Then, by changing the base-pair spacing between the two different elements, their bends can be brought into and out of phase with each other. Figure 2 illustrates how small elements of curvature can either cancel or add constructively, depending on whether they are in the same or opposite directions.

Runs of homopolymeric dA·dT 5 to 6 base pairs long, or A tracts, provide a standard against which other bends can be judged. Even though the structural basis for DNA bending at A tracts is still unresolved (*5*), the global features of the bend are well established (*4*, *6*): A tracts 5 to 6 base pairs long induce a bend of 18° ± 10% when inserted into generic B DNA; the bend direction is toward the minor groove in a coordinate frame near the center of the A tract. Because the regions of neutralized DNA charge should also induce a bend toward the minor groove (because of the missing electrostatic repulsion there), one expects that the A tract and neutral patch bends should reinforce each other when they are separated by one helical turn, or about 10 base pairs. This prediction agrees with the experimental observations. Furthermore, the overall curvature resulting from the two bends combined is about the same as that of two A tracts when the electrophoresis medium contains 45 mM $tris^+$, so the neutral patch bend is about 20°. In a medium containing multivalent ions, which is more like the intracellular environment, the bend is reduced to 7° to 11°.

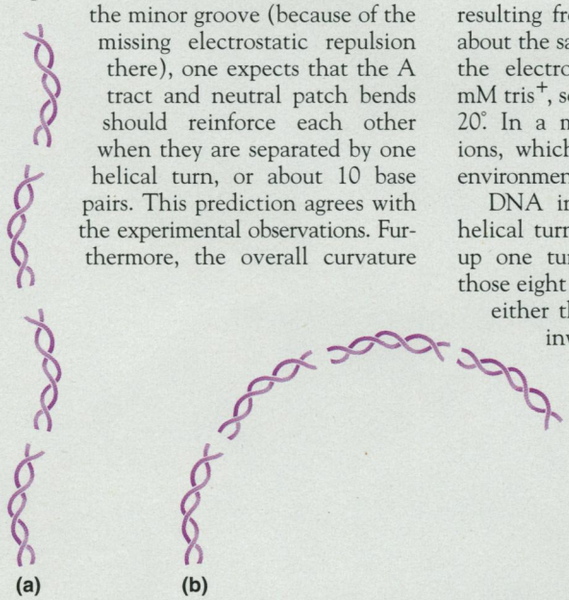

Fig. 2. Multiple bends. Repetition of helical elements with small curvature can produce nearly straight (**a**) or strongly bent (**b**) overall structures, depending on whether the bends are on opposite sides of the helix (a) or on the same side (b).

DNA in a nucleosome contains eight helical turns in the 360° bend that makes up one turn of the solenoidal helix. In those eight turns, there are 16 loci at which either the major or minor groove faces inward, so the average bend at each locus is about 22°. Hence, the 7° to 11° curvature induced by the neutralizing DNA charges at neighboring positions across the minor groove provides a third to half of the curvature of DNA at such a site in the nucleosome. Given the quadratic dependence of the bending energy on curvature, reducing by a third the amount of additional bending required

The author is in the Sterling Chemistry Laboratory, Yale University, 225 Prospect Street, New Haven, CT 06511, USA.

to reach nucleosomal curvature reduces by more than a factor of 2 the required extra energy. Hence, one can conclude that restoring the balance of forces in an asymmetrically neutralized DNA requires compression of the neutralized face, resulting in curvature that is energetically significant on the scale of DNA packaging.

Not unexpectedly, charge neutralization does not account for all of the energy needed to bend DNA in nucleosomes. The interaction of DNA with positively charged nucleosomes (Fig. 1) releases counterions that were closely associated with the highly charged DNA molecule. Record and Manning and their co-workers (*7*) emphasized the importance of the entropy gain of counterion release in the overall thermodynamic balance of protein-DNA interactions. This thermodynamic factor clearly helps drive the bending of DNA in nucleosomes.

Among the questions that remain is the extent of curvature induced when the neutralized charges are placed opposite each other across the major rather than the minor groove. Because the DNA major groove is the wider of the two, one might expect less reduction in electrostatic repulsion, and hence less bending, at such a locus. It would also be useful to examine the quantitative consequence for the bend angle estimation of taking accurate account of the displacement from the center of the A tract, by ~0.5 to 1 base pair, of the coordinate frame that defines a minor groove–directed bend (*4*, *6*).

Comparison of the new results with the quantitative theory of Manning and coworkers (*2*) is complicated by a prediction in the theory that does not seem to appear in the experimental findings. The theory models DNA as an elastic rod to which the unbalanced electrostatic force contributes an eccentric load, which has both compressive and bending components. Longer DNAs have a larger lever arm for the bending moment and hence have greater curvature, or a smaller radius of curvature. However, there is nothing in the experiments to indicate anything but a constant curvature, a constant bend per neutral patch, independent of the overall DNA length. It will be of interest to revisit the theoretical analysis in search of the origin of this apparent discrepancy.

Finally, it should not be thought that the phenomenon of charge neutralization contributes to all protein-induced DNA bends. In some cases, such as the interaction of TATA-binding protein (TBP) with the TATA element in promoter DNA, the DNA is strongly bent away from the DNA face that provides the primary contact with protein (*8*). Clearly, the catalog of mechanisms and associated energetics by which proteins bend DNA is not yet complete.

References

1. A. D. Mirzabekov and A. Rich, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1118 (1979).
2. G. S. Manning, K. K. Ebralidse, D. D. Mirzabekov, A. Rich, *J. Biomol. Struct. Dyn.* **6**, 877 (1989).
3. J. C. Marini, S. D. Levene, D. M. Crothers, P. T. Englund, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7664 (1982); H.-M. Wu and D. M. Crothers, *Nature* **308**, 509 (1984).
4. D. M. Crothers and J. Drak, *Methods Enzymol.* **212B**, 46 (1992).
5. D. Goodsell, M. Kaczor-Grzeskowiak, R. E. Dickerson, *J. Mol. Biol.* **239**, 79 (1994); R. E. Dickerson, D. S. Goodsell, S. Neidle, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3579 (1994); T. E. Haran, J. D. Kahn, D. M. Crothers, *J. Mol. Biol.* **244**, 135 (1994).
6. D. M. Crothers, T. E. Haran, J. G. Nadeau, *J. Biol. Chem.* **265**, 7093 (1990).
7. M. T. Record Jr., C. F. Anderson, T. Lohman, *Q. Rev. Biophys.* **11**, 103 (1978); G. S. Manning, *ibid.*, p. 179.
8. Y. Kim, J. H. Geiger, S. Hahn, P. B. Sigler, *Nature* **365**, 512 (1993); J. L. Kim, D. B. Nikolov, S. K. Burley, *ibid.*, p. 520.