Intrinsically Bent DNA

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Intrinsically bent or curved DNA molecules result when special base sequences or structural motifs are repeated in phase with the DNA helical repeat (&approx;10.5 bp/twist). This places the recurrent elements along the same side of the double helix so that small bends associated with them add constructively to generate a large global curvature. Many base sequences can impart systematic curvature to DNA, but most such bends are small compared with the special effect produced by runs of homopolymeric dA-dT base pairs ("A-tracts"), each tract about half a helical turn long and repeated at 10–11-bp intervals.

Bent DNA was discovered by the laboratories of Englund and Crothers (1) during study of a minicircle DNA from the kinetoplast body of Leishmania tarentolae, in which appropriately phased A-tracts occur prominently (1, 2). The anomalous gel mobilities shown by bent DNA molecules have provided crucial experimental insight into the origin and nature of DNA bending. Application of early theories describing gel mobilities for molecules undergoing reptational migration (3, 4) leads one to expect slower motion for bent molecules because they have a shortened end-to-end distance. This criterion has proved to be a reliable guide to experimental properties, even though it ignores significant theoretical issues (5).

A critical experiment for diagnosing the presence and location of DNA bends has been comparison of the electrophoretic properties of circularly permuted DNA molecules, all of the same length but each having the bend at a different position. In the first application of this concept, Wu and Crothers (6) were able to identify a locus containing phased dA-dT tracts which provided the primary source of bending in Leishmania kinetoplast minicircle DNA. Since that time, phased dA-dT tracts have been identified in various gene regulatory regions (7–10), but the biological function of intrinsically bent DNA remains uncertain.

Theoretical Models for DNA Bending

Initial formulation of models for DNA bending was prompted by recognition that DNA must be bent for packaging into nucleosomes (11–13). Zhurkin et al. (14) concluded on the basis of conformational energy calculations that bending in nucleosomes should occur by roll every 5 bp, alternately toward both major and minor grooves, in preference to tilting because they have a shortened end-to-end distance. This criterion has proved to be a reliable guide to experimental properties, even though it ignores significant theoretical issues (5).

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The model-building studies of Arnott, Wells, and collaborators (16) focused on an abrupt change in the DNA helix axis direction at the junction of A- and B-DNA helices. The bases in the two forms have different angular orientations relative to their respective helix axes, parallel stacking of the base pairs at the junction causes the two helix axes to be non-parallel. The locus at which the axes meet is called a "junction bend" in the model for curvature resulting from A-tracts (6, 17).

It should be emphasized that the wedge and junction models are not necessarily incompatible. For example, strong inclination of the base pairs in an A-form DNA helix is necessarily accompanied by appreciable roll between them; consequently, curvature induced by placing a short A-form structure between two B-DNA helices can be viewed as arising either from the two junction bends or from the series of (roll) wedges between adjacent base pairs in the A-form segment. In the first case, one views the A-DNA segment as a whole, but in the second case the handedness of helix axes whose direction deviates from that of the adjacent B-DNA segments. In the second case, the emphasis is on a local axis (defined as perpendicular to the base pair plane), which is deflected in a series of steps.

It is our view that the wedge model is the more general one, since any curving helix can be described by combining helical rotation with an appropriate series of wedge angles. However, the more restrictive and hence informative junction model is to be preferred where it actually applies. Specifically, the junction model is appropriate when evidence exists for a distinctive helical structure formed by the base sequence in question and when there are cooperative effects in nucleating this special structure within a segment of B-DNA; both of these tests are met for DNA bends that occur at dA-dT tracts. Cooperative induction of the special structure weakens the applicability of the wedge model because the dinucleotide-based wedge angles derived for B-DNA cannot be relied on to predict the properties of the altered structure and hence of the bend in a longer tract.

Models for the structure of poly(dA)-poly(dT) based on fiber diffraction (18, 19) share the common feature of negative roll between adjacent base pairs, which yields base pairs with negative inclination relative to the overall helix axis. In 1986, Koo et al. (21) pointed out that application of this characteristic predicts a bend oriented toward the minor groove at the center, or toward the 5'-strands at the ends, of the dA-dT tract (Fig. 1); both the direction and approximate magnitude of the bend as subsequently observed have been in general agreement with the model. However, uncertainty remains because crystallographic structures of oligonucleotides con-
A direct and clear evidence for the presence of a static bend is that the ligated oligomers containing repeated (dA·dT)₆ A-tracts at various phasings revert to a more canonical B-DNA form at lower temperatures. The effect of elevated temperature is to reduce DNA bending (1, 28). By 60 °C the anomaly effectively disappears (28), presumably because the special structure of dA·dT tracts reverses to a more canonical B-DNA form.
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Tures more complex dependences are observed (21, 28, 29), largely because the increased tendency to anomalous structure at low temperature may be compensated by the effect of temperature on the DNA helical screw (30), which can affect the phase match between A-tracts and the helix repeat.

Ionic conditions have an appreciable but not dramatic effect on apparent DNA curvature. Increasing NaCl concentration decreases the gel migration anomaly (2, 28), whereas the migration anomaly of most, but not all, sequences is increased by adding divalent ions (2, 28, 31–33). As with the case of temperature effects, it is not yet possible to deconvolute these effects fully into influence on actual bending, influences on the DNA helix screw, and possible effects on the calibration function between gel mobility and DNA curvature.

The Direction and Magnitude of Bending

In 1986 Koo et al. (21) showed that DNA bend direction is nearly unaffected by interchanging the T and A strands in a (dA·dT)n tract. Hence the net bend has an approximate dyad axis that runs between the major and minor grooves at the center of the tract, and the bend must therefore be primarily directed toward the major or minor groove at the center. Addition of divalent ions has only a minor effect on bend direction, since the mobility anomalies measured by Diekmann (28) for mixed A and T tracts, compared with pure A tracts, imply only small differences in relative curvature over a wide range of Mg²⁺ concentration.

The remaining ambiguity in A-tract bend direction (i.e. major versus minor groove compression) was resolved in 1987 by Zinkel and Crothers (34), who measured the curvature direction relative to the bend induced when DNA wraps around CAP protein. They concluded that the A-tract bend direction is equivalent to minor groove compression at the center of the A-tract. Viewed from the global perspective of the overall bend, this is geometrically equivalent to (negative) tilt of the helix axis toward the sugar-phosphate backbone at the 3'-end of the A- and T-tracts (Fig. 1). This conclusion is consistent with comparative studies of the direction of the bend induced when the backbone tilts away from an extra or "bulged" base inserted on one strand (35).

Hagerman (36) explored the properties of adjoining (dA·dT)n tracts and found that oligomers of the form (A₃T₃N₃)n exhibit extremely anomalous electrophoretic mobility, in sharp contrast to molecules of the form (T₃A₃N₃)n, which show virtually normal mobility. Although these results were unpredicted, both the wedge and the junction models could subsequently be adjusted to account for them (37, 38).

Estimates of the extent of bending produced by phased A-tracts have varied by more than a factor of 2, from about 11° (39) to about 28° (31). The hydrodynamic experiments of Levene et al. (40) gave an estimate of 9° for each junction or about 18° total. This result is in agreement with the electron microscopic observations of Griffith et al. (41) combined with recent computer simulation of cyclization kinetic experiments (42). Hence, with a total bend of 18° for (dA·dT)n, there must, in any model, be about a 9° difference in the average base pair inclination between the A-tract and adjacent B-DNA.

Cooperativity in A-tract Structure

The local structure of DNA can in principle be governed either by nearest neighbor effects in which the conformation of each base pair step is independent of that in adjacent steps or by longer range phenomena. Persuasive evidence now exists that as an A-tract is lengthened there is a cooperative switch from B-DNA structure to one more characteristic of poly(dA)·poly(dT). The properties of multimers of the series TₙAₙNₙ₋ₙ (n = 1–4) studied by Haran and Crothers (43) imply cooperative structural effects. Sequences with n = 1–3 migrate slowly in gel electrophoresis, whereas the TₕAₙNₙ multimers migrate almost normally (due to offsetting bends associated with segments Tₙ and Aₙ). Neither the wedge nor the junction model can account for the abrupt change in mobility observed in this series unless one assumes a cooperative structural transition in the Tₙ segment as n increases from 3 to 4.

NMR spectroscopy provides direct evidence for cooperative changes in helix structure as the length of the A-tract is increased. From measurements of imino proton exchange rates Leroy et al. (44) observed that A-tracks with n ≥ 4 show anomalously long lifetimes which increase further for n = 5, 6. Nadeau and Crothers (45) examined the NMR properties of a series of molecules containing A-tracks of different lengths (n = 2–9). Thymidine imino protons in short tracts (n = 3) tend to have chemical shifts like those characteristic of B-DNA, as do the imino protons near the 5'-end of an A-tract of any size. The 5'-end of the A-tract has much less tendency to hold the A-tract in the conformation characteristic of B-DNA.

Crystal Structures

Attempts to reveal the molecular details responsible for sequence-directed bending have led to structural determinations of several A-tract-containing duplexes by single crystal x-ray methods (46–49). The propeller twist of the A·T pairs in these structures is so pronounced that the C6 amino group of adenine, in addition to participating in the normal Watson-Crick pairing scheme, may form a second hydrogen bond with the O4 atom of the 3'-neighboring thymine. The resulting network of bifurcated H bonds connecting adjacent base pairs within the A-tract has been suggested (46, 47) as the basis for the apparent stiffness of poly(dA)·poly(dT) (50) and as a medium for the propagation of length-dependent changes in A-tract structure. However, the anomalous electrophoretic migration (25, 26) and long proton exchange rates (44) of imino-containing A-tracks in solution suggest otherwise; since the number of potential bifurcated hydrogen bonds is reduced in these sequences, their contribution to A-tract structure and bending is dispensable.

Unfortunately, crystallographic structures also leave ambiguity concerning the source and direction of A-tract curvature of unconstrained molecules in solution. Duplexes in the crystalline lattice are bent in a direction roughly perpendicular to the overall curvature deduced from comparative electrophoretic mobility studies (21, 34). Moreover, DiGabriele et al. (48) have provided clear evidence that A-tract curvature in the crystal is due primarily to the intermolecular packing constraints of the crystal lattice. Models for DNA bending have nevertheless been proposed based on the assumption that the high propeller twist and straight local helix axis characteristic of crystalline A tracts persist in solution (39, 46, 51).

Solution Studies

Despite the absence of reliable three-dimensional structures of A-tracks in solution, certain revealing features have been established. Short A, tracts (n < 7) lack regions of true poly(dA)·poly(dT)-like conformation and display non-uniform structure characterized in part by a progressive reduction in minor groove width in moving from the 5'- to the 3'-end of the A-strand (45, 52–54). In longer A, tracts (n ≥ 7), the minor groove narrows gradually over the first three base steps and then remains fairly constant over the remainder of the tract (45). In contrast, the GC-rich regions flanking the A-tracks feature a wider minor groove typical of canonical B-DNA; thus periodic alternation of A-tracts and GC-rich se-
quences leads to sinusoidal variation in minor groove width as detected by hydroxyl radical cleavage patterns (52). In the crystalline A-tract structures, the large propeller twist of the A-T pairs narrows the minor groove, in apparent qualitative agreement with solution results.

However, groove width is also governed by factors apart from propeller twist including base pair inclination and displacement of the base pairs from the local helix axis. These latter two quantities, both negligible in crystalline A-tracks, are substantial in models for poly(dA).poly(dT), for which substantial experimental support exists. The large propeller twist of the A-tract results in a considerable inclination of the A-T base pairs (58), which cannot form in GC-rich segments, key interproton distances are more consistent with poly(dA)-poly(dT) models featuring substantial base pair inclination rather than high propeller twist (45). Thus it appears that A-tract structure in solution differs significantly from that observed in the crystal form.

A Unifying Model

As noted above and illustrated in Fig. 1, the direction and magnitude of bending are quite accurately predicted if one assumes that base pairs within A-tracks are inclined relative to the helix axis much as they are in models of poly(dA)-poly(dT), for which substantial experimental support exists. Energy calculations on poly(dA)-poly(dT) (58) indicate that the stacking energies for A-T pairs in this conformation are suboptimal but also that this seemingly unfavorable arrangement promotes formation of a network of hydration (59, 60) in the minor groove (linking Thymine 02 and Adenine N3 atoms on opposite strands) that more than compensates for the lost stacking energy. Removal of this water spine by increased temperature or organic solvents should reduce bending (61) by freeing the A-T pairs to adopt a more favorable base-stacking arrangement in which they are perpendicular to the helix axis (55). The hydration network cannot form in GC-rich sequences because the guanosine 2-amino group intrudes into the minor groove. According to the model, cooperativity effects should arise at least in part from the relative instability of the water spine in short (n = 2-3) A-tracks; once the A tract has reached a length of 4 this nucleation effect is largely overcome. In addition, since minor groove hydration is thought to be disrupted by TpA but not ApT steps, a contiguous array of inclined A-T pairs may run across ApT but not TpA steps. Thus one expects the A and T steps in sequences A\textsubscript{3}T\textsubscript{3} to act in concert as a single cooperative unit, whereas those in sequences T\textsubscript{3}A\textsubscript{3} should behave independently. NMR (44) and comparative gel mobility (38, 43) studies show this to be the case.

We caution that Fig. 1 represents an idealized model for bending, wherein the conformation of all AA/TT base steps is identical and the bends are exclusively in the direction of tilt at the junctions. All models that seek to explain DNA bending require a difference in base pair inclination between A-tract and B-DNA structures; we attribute the effect primarily to negative base pair inclination in the A-tract, which in turn is probably induced by strong minor groove hydration there. We expect, however, that significant positive or negative inclination components can exist in the intervening B-DNA segments and that these will be found to cause modulation of DNA curvature in response to sequence alterations in the intervening B segments.
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