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Helical parameters of DNA do not change when DNA fibers are wetted: X-ray diffraction study

(DNA structure/B-form DNA/pitch/residues per turn)

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ABSTRACT **We have measured the helical parameters of DNA in concentrated solutions by x-ray fiber diffraction methods. Fibers of the sodium salt of DNA were swollen with water within capillaries; the capillary served to limit water uptake, slowing dissolution. Samples containing up to 80% water gave essentially a B-form diffraction pattern and had virtually the same helical parameters [9.91 base pairs per turn (SD = 0.14); 3.34 Å axial rise per residue (SD = 0.019)] as did the initial fibers [9.95 base pairs per turn (SD = 0.15); 3.33 Å axial rise per residue (SD = 0.015)]. Hence, under highly solvated conditions in which the interactions between molecules should be greatly decreased, DNA maintains its classical B-form structure.**

The high-humidity or B-form of DNA has been widely assumed to be the structure of DNA present in solution. This familiar double-stranded helical structure has a pitch of 34 Å and an axial residue repeat distance of 3.4 Å, corresponding to 10.0 base pairs per turn of the helix (1–3). Recently, several authors have suggested that this structure, which was determined in DNA fibers, is not retained in solution. A theoretical study led Levitt (4) to suggest that the helical parameters of DNA in solution correspond to 10.6 base pairs per turn with attendant major changes in sugar conformation and base-pair tilting. Griffith (5) has proposed a structure with 10.5 base pairs per turn to rationalize the linear dimensions of DNA as visualized by electron microscopy. Wang (6) has inferred a value of 10.4 base pairs per turn based on the effects of oligonucleotide insertions on the topological properties of closed circular DNA.

As well as being of intrinsic interest, the values of the helical parameters have important implications for DNA supercoiling in nucleosomes. We have therefore attempted to extend to DNA solutions the technique that was used originally to measure the helical parameters of DNA in fibers—namely, x-ray fiber diffraction. Generally, this approach has been applied to fibers because of the ease of obtaining the required parallel molecular orientation. However, there is no reason why it could not be applied to solutions if sufficient molecular orientation could be maintained over the period necessary to collect the diffraction data and if the solvent scattering did not interfere. We report here such measurements on DNA in concentrated solutions. Fibers of DNA were swollen with water within capillaries; the capillary limited the water uptake to controlled ranges and greatly retarded the dissolution of the fiber. The lack of changes in the diffraction pattern upon wetting of the fibers indicates that a model with the original helical parameters and structural characteristics of B-form DNA is still the most reasonable candidate to represent DNA in our concentrated DNA solutions.

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MATERIALS AND METHODS

Salmon sperm DNA (Calbiochem) was purified essentially as described by Neville and Davies (7). The following steps were done at 0–5°C. A solution (300 mg of DNA in 60 ml of 0.1 mM EDTA, pH 7) was centrifuged for 1 hr at 27,000 × *g*. The supernatant fluid was decanted and dialyzed for 2 days against 2 liters of 1 M NaCl; then the DNA was precipitated with an equal volume of isopropanol, wound up on a rod, and soaked while on the rod in two changes (85 ml each) of 70% ethanol for 30-min periods. The fibrous material was dried under reduced pressure. The dried DNA was redissolved (at 1 mg/ml) in either 1 or 10 mM NaCl and then centrifuged for 16 hr at 35,000 rpm in the Spinco SW 40 rotor. The pellet was briefly drained and stored at 5°C until used to form fibers. The DNA content of pellets from several preparations at either salt level ranged between 1 and 3% (based upon the absorbance when weighed portions of pellets were redissolved, assuming $A_{260} \approx 20$ for 1 mg/ml).

DNA Fibers. A portion of pelleted DNA was suspended between two supports (either two Chromel wire loops or two segments of thoroughly washed toothpicks). The DNA gel was allowed to dry at ambient conditions with frequent small extensions of the space between the supports. Fibers were 100–300 μm in diameter and 2–5 mm long.

Fibers with such diameters and possessing good molecular orientation were only obtained from DNA pelleted from solutions over a limited range of salt concentration (1–10 mM NaCl). Below these salt concentrations, the fibers could not be formed. Above these salt levels, the molecular orientation in the fibers was inadequate, probably due to deposition of salt crystals within the fibers. The DNA is concentrated about 30- to 100-fold upon drying into fibers, so this range of NaCl concentrations yields up to about 6% NaCl by weight in the fibers. The fibers containing 6% NaCl gave B DNA patterns at 98% relative humidity (Fig. 1*A*) or A DNA patterns at 79% relative humidity (Fig. 1*B*) as expected (8). The B DNA pattern was collected from a number of independent fibers to help assess the variation expected in the studies on wet DNA, and the data will be presented below. The salt content of these fibers was a potential variable in the estimation of helical parameters from wetted DNA because, necessarily, the DNA was dissolving into relatively concentrated salt solutions (0.25–1 M NaCl, depending upon the degree of wetting). Accordingly, we ran a second series of wetted DNA samples from fibers that contained about 0.6% NaCl and which should yield 0.02–0.1 M NaCl upon wetting. These samples containing less salt turned out to have virtually the same helical parameters as did the higher salt samples (see below). (Fibers made under the lower salt condition gave a mixture of A and B forms at 98% relative humidity and so no attempt was made to get helical parameters from these fibers before wetting.)

Abbreviations: p, axial residue repeat distance; P, pitch.

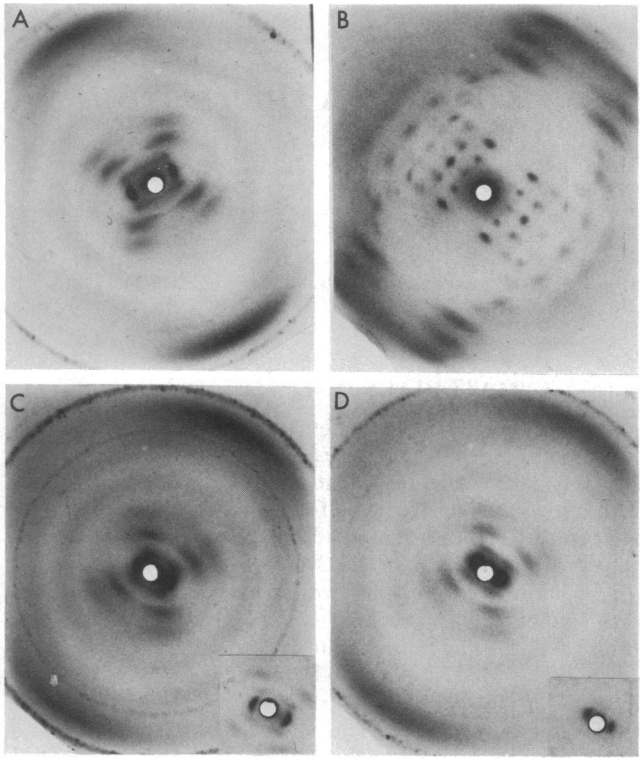

FIG. 1. X-ray diffraction photographs of DNA as a fiber or in solution. (*A* and *B*) Diffraction patterns from fibers at 98 or 79% relative humidity, respectively, yielding typical B- and A- type patterns. (*C* and *D*) Diffraction patterns from wetted DNA samples. The sample in *C* is from the low-salt series and has an equatorial spacing of 24.7 Å; that in *D* is from the high-salt series and has an equatorial spacing of 38.9 Å. In all cases the direct beam is essentially normal to the fiber axis or capillary. The long axis of the capillary or fiber is tipped slightly from vertical relative to this page. *Insets* (in *C* and *D*) are shorter exposures to demonstrate the changes in the equatorial spacings.

Wet DNA Samples. A column of water 0.5–1 cm long was introduced into one end of a thin-walled Lindemann lead glass capillary (internal diameter, 0.2–0.5 mm) and that end of the capillary was sealed with wax. A DNA fiber was introduced from the other end and pushed down the capillary until about 10% of its length was immersed in the column of water. The wetted end of the fiber rapidly swelled until it formed a porous plug between the water and the rest of the fiber. The open end of the capillary was then sealed with wax. Over the course of several hours, the rest of the fiber gradually swelled in diameter to fill the capillary. In some cases the uptake of water caused a separation of the fiber from direct contact with its water supply and further swelling was slow. Much slower swelling, up to several days, was induced by sealing a fiber in a capillary with a water column, but without allowing the fiber to contact the water; the orientation was no better preserved by this protocol. Swelling was also induced by rapidly immersing a fiber in water along its total length, so that the entire fiber swelled to fill the capillary within a few seconds; molecular orientation was poorer after this rapid swelling.

X-Ray Pictures. Copper Kα radiation was obtained from a Norelco fine focal spot x-ray tube. Diffraction patterns were recorded at room temperature in a Norelco microcamera modified to accommodate a specimen-to-film distance of about 34 mm. The exact distance for each sample was judged by the diameter of the 3.035-Å diffraction ring from a calcite coating applied both to fibers and to the outer surface of the capillaries used for the wetted DNA samples. The spacings of the five most intense diffraction rings of the calcite standard agreed, to within 0.1%, with the values determined by the National Bureau of Standards (9) [still photographs taken with Supper precession camera, 6-cm specimen-to-film distance; calibration checked with powdered NaCl (10)]. Humidity was controlled for fiber pictures by continuously flushing the camera with a stream of helium that had been passed through the appropriate saturated salt solution [$KClO_3$ or NH_4Cl for 98 or 79% relative humidity, respectively (11)], as well as by the presence of a dish of the salt solution within the camera. Dry helium was used to flush the camera when sealed wet DNA samples were photographed. The exposures to determine helical parameters were 3–6 hr long. Immediately before and after such an exposure, a 30-min exposure was taken to measure the spacing of the much more intense equatorial reflection.

Distances on the films were routinely measured with a comparator (Nikon, Shadowgraph model 6). This device projects a magnified image of the x-ray picture upon a screen. A micrometer screw drives the table holding the picture so that the image moves relative to a reference line on the screen. The distance moved is measured by the changes in micrometer settings. Scanning of several films with a Joyce-Loebl densitometer yielded consistent results.

The diameter of the diffraction ring of the calcite standard was measured on each picture at three or more places and the mean value was used to determine the specimen-to-film distance. Measurements of layer line spacings were generally made between related layers—i.e., from layers 1 to −1 or from layers 10 to −10. The film distance between layers 10 and −10 was taken along the meridian between the points of estimated maximal intensity of those layers. The distance from the equator to layer 10 or −10 was unchanged when fibers were tilted to 77° from the incident x-ray beam; for this reason, intensity on these layers from untipped samples was assumed to be substantially meridional in origin and to arise because of partial disorientation of the molecules. To verify this interpretation, a precession picture was taken of a fiber (μ = 16°, specimen-to-film = 6 cm, calcite standardization; fiber sealed in capillary with water); the spacing of the 10th layer was 3.34 Å, in excellent agreement with the values determined in the microcamera (see Table 1). The Bragg distance of this layer then gives the residue repeat distance (p) of the helix (12).

Layers 1 and −1 were generally flat, and the distance between them did not vary when measured on or off the meridian. In practice, the reference line of the comparator was centered along the total observable length of the layer and the distance required to move the reference line to lie along the comparable portion of the related layer was noted. After five measurements were taken in this fashion, the film was rotated 180° so that the direction of movement was reversed and a further five values obtained. This whole process was repeated once more and the values were averaged to get the layer line spacing. This spacing corresponds to the pitch (P) of the helix (12). Several of the samples of wetted DNA showed a partial blurring of the outer edges of the first layer, presumably due to disorientation; for these samples, the portion of the layers nearer to the meridian was used to obtain the pitch.

RESULTS

X-Ray Diffraction Patterns of DNA in Fibers. Natural DNA in fibers can generally be induced to adopt any of three different secondary structures (A, B, and C forms), each with a distinctive x-ray diffraction pattern (2, 13, 14). The secondary structure that is present is dependent upon the species of cation, the salt concentration in the fiber, and the relative humidity with which the fiber is equilibrated (8). The sodium salt of DNA

was chosen for the present study because, in fibers at high humidity and in the presence of adequate levels of salt, sodium DNA forms a noncrystalline B-form DNA structure. We thought that estimation of the helical parameters from such fibers would be an excellent control for estimation of such parameters from the pattern of wet DNA which, as described below, is essentially that of B DNA. A typical fiber pattern is shown in Fig. 1*A*. The estimation of helical parameters from such patterns and their comparison with similar data from wetted DNA will be discussed below.

Background for Interpretation of the X-Ray Patterns. Two parameters are necessary to define the basic repeat pattern of a helix. The parameters usually chosen are P which is the rise along the helix axis corresponding to one complete turn of the helix and p which in the present case is the rise along the helix axis per base pair. The helix may also be described by the number of residues per pitch length, P/p. In principle (12), P and p can be obtained directly from x-ray diffraction photographs of helical molecules, if the molecules have a sufficient degree of parallel alignment. The parallel alignment causes the diffraction pattern to be confined to regularly spaced layers; P is simply the reciprocal of this spacing between layers (or some integral multiple thereof if the molecules have rotational symmetry about their helix axes). The other parameter, p, is equal to the reciprocal of the spacing for the first layer upon which a truly meridional reflection occurs (layer 10 for B DNA). As customary, "meridional" and "equatorial" refer to vertical and horizontal directions, respectively, through the center of photographs for which the fiber axis is oriented vertically. The large amounts of solvent in some of our samples should not interfere in obtaining accurate helical parameters as long as the solvent molecules are disordered. Such disordered materials will make only minimal contributions on the layer lines associated with the DNA helix.

The intensity on the equator of the photograph reflects the side-by-side arrangement of the molecules. In the present case, all of the DNA samples give a single strong reflection on the equator. The position of this spot is a measure of the spacing between molecules. Molecules such as DNA, which are roughly circular in cross section, would be expected to pack in a hexagonal net in the absence of specific crystalline interactions (see chapter 6 of ref. 15). This is especially likely when the DNA is wetted. The equatorial spot from such an array would then correspond to the (10) reflection from a hexagonal net. (The Bragg spacing of this reflection is $\approx 1/1.155$ of the actual spacing between the helix axes of the molecules.)

X-Ray Diffraction Pattern of Redissolved DNA Fibers. The diffraction patterns were collected from a series of sodium DNA fibers after they had been allowed to imbibe graded amounts of water. The most striking feature of these patterns (Fig. 1 *C* and *D*) is that they were basically the B DNA pattern, irrespective of the extent of water uptake, of whether the DNA in the original fiber was in the A or B form, and of the salt levels in the fibers. Pictures taken 1–2 weeks after water uptake showed the same basic B DNA pattern but with increased molecular disorientation. Pictures taken to look for non-B DNA reflections [long exposure times, short specimen-to-film distance (14 mm)]; no such reflections were seen.

The helical parameters were estimated from a number of fibers before and after wetting. The data are summarized in Fig. 2. Average values for the helical parameters are listed in Table 1. Within the limits of the data, there was no change in P, p, or P/p when DNA fibers were wetted to varying extents. The precision of these results is indicated by the standard deviations in Table 1. Variation in the values of P limits our estimates of the number of residues per turn of the helix. The mean

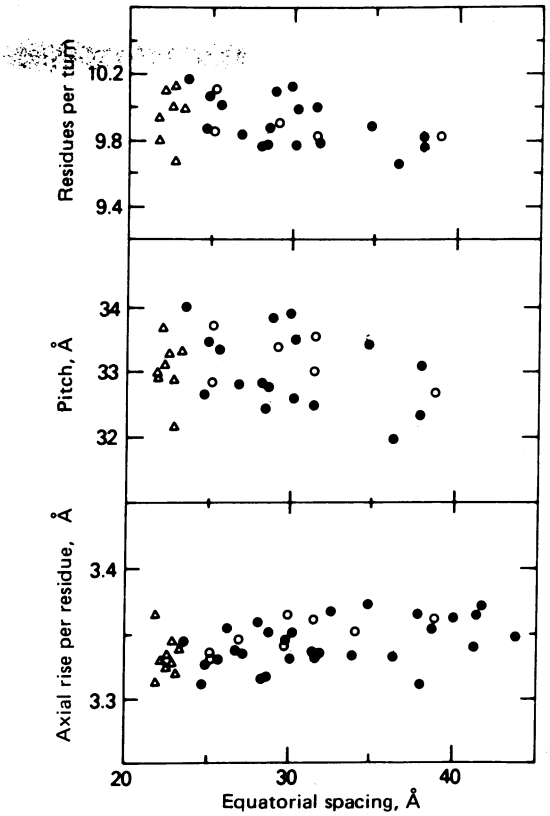

FIG. 2. Helical parameters of sodium DNA in fibers or in solution. All the values measured for the helical parameters are plotted here. The actual spacing between the helix axes of the molecules is 1.155-fold larger than the equatorial spacing shown, assuming hexagonal spacing (see text). △, Fibers at 98% relative humidity; ●, wetted DNA samples, low-salt series; ○, wetted DNA samples, high-salt series.

± 2 SD is 9.6–10.2 residues per turn for fibers or for wetted samples.

A possible systematic error could arise from the influence of disorientation upon the intensity distribution along the meridian for layer 10. Disorientation will shift some of the intensity to larger spacings on the film, which may cause an underestimation of p. A densitometer scan along the meridian through this layer showed no obvious asymmetry, suggesting that underestimation would be minor in extent. The largest value p could have is about 3.38 Å based on the Bragg spacing of the inner boundary of layer 10. We are left then with somewhat smaller values for p and P than were reported previously for B form patterns, although the ratio P/p is similar to that reported earlier. Our sodium DNA fibers have p = 3.33 Å and P = 33.1 Å. Langridge *et al.* (16) found P = 33.6, 33.7, and 34.6 Å for several types of lithium DNA fibers and P = 34.6 Å for a semicrystalline (hexagonal) type of sodium DNA fiber (P/p = 10 in all cases).

Table 1. Helical parameters of DNA as fibers or in solution

Sample	Helical parameters		
	Pitch (P), Å	Axial rise/ residue (p), Å	Residues, turn (P/p)
Fiber	33.1 (0.48)	3.33 (0.015)	9.95 (0.15)
Low-salt solution	33.0 (0.58)	3.34 (0.019)	9.91 (0.14)
High-salt solution	33.1 (0.37)	3.35 (0.016)	9.91 (0.13)

Helical parameters are unweighted averages of all the data of Fig. 2. Values in parentheses are SD.

Although basically of the B DNA type, the pattern did change in several respects upon wetting. The intensity and spacing of the equatorial spot changed relative to that in fibers (Fig. 1 *A* vs. *C* and *D*). Also, there are changes in the pattern that are indicative of an increase in molecular disorder. The interpretation of these changes will be discussed below.

DNA Molecules Become Separated as the Fibers Redissolve. The fibers became swollen in a roughly homogeneous fashion as judged by their appearance under polarized light. Because we wished to infer properties of DNA in solution from the x-ray diffraction patterns of these expanded fibers, it was important to demonstrate that the molecules became uniformly immersed in the medium as isolated molecules and not, for example, as bunches of molecules separated by regions of solvent. Before wetting, the DNA fibers gave a single sharp equatorial reflection which is a direct measure of the intermolecular spacing as noted above. The relative increase in this spacing upon wetting was equal, within 20%, to the relative increase in the diameter of the fiber upon wetting, implying that the molecules were indeed uniformly surrounded by solvent. In relatively short exposures, the equatorial reflection was still sharp after swelling. Hence, these results indicate that, upon swelling, the average intermolecular distance increased in accord with the expectations from uniform immersion of the molecules in the solvent. There was a striking increase in the intensity of the equatorial reflection. This increase is qualitatively the effect predicted for parallel arrays of cylindrical fibrils when they expand from being in contact to having interfibril spaces of the order of their diameters (see chapter 6 of ref. 15).

Disorientation Increases Upon Wetting of DNA. A fiber composed of polymeric molecules often has extensive parallel orientation between its molecules with respect to their long axes. Beyond this, molecules within noncrystalline fibers may show continuous shift and rotation—i.e., random mutual displacements along their long axes and random azimuthal orientations about their long axes. The diffraction pattern of our sodium DNA fibers is consistent with all of these characteristics. Wetting of the fibers invariably led to more or less severe changes in the patterns that are consistent with more or less of a loss of the parallel orientation. It should be emphasized that, no matter what the degree of disorientation, each of more than 50 wet DNA patterns was readily recognizable as a B DNA pattern. Furthermore, in all cases the changes in the patterns due to disorientation were basically of a single kind, although varying widely in degree.

Generally, the more water that was absorbed when the fiber was wetted, the greater was the disorientation. Indeed, it has been impossible to get adequate patterns by our present techniques when the volume of the fiber increased upon wetting by more than about 4-fold, and many samples at lower extents of water uptake did not retain sufficient orientation to be useful for estimating helix parameters. A further, more gradual, loss of orientation occurred after the uptake of water was complete. This was particularly obvious in the samples containing higher salt concentrations; with these the quality of the patterns noticeably degraded within several hours after wetting. Lower salt samples were more stable and useful pictures were often taken for periods of several days.

Vainshtein (chapters 6 and 7 of ref. 15) has discussed the changes in diffraction patterns of polymers expected in response to various types of molecular disordering. We have interpreted our patterns in terms of his discussion. The molecules in fibers would be expected to have continuous shift and rotation relative to each other, which will ensure a continuous transform on all layer lines except the equator. As the molecules become separated from each other, the packing considerations that made them lie in parallel arrays become less important and the helix axes may reasonably be expected to adopt nonparallel orientations relative to each other. Whether this occurs between individual molecules or between groups of molecules, the layer lines will be transformed, in the extreme case, into rings with a sharp inner edge and a more gradually decreasing outer edge. This is just what we observe in our disoriented wet DNA patterns. If this interpretation of the major disorientation in wetted samples is correct, it should be possible to estimate the layer spacings in disoriented samples by the Bragg distance of the sharp inner boundary of the arc or ring. In practice, the exact position of this boundary could not be estimated accurately enough to be of use in determining P.

DISCUSSION

The original determination of the helical parameters of DNA was made from the wide-angle x-ray diffraction pattern from DNA fibers. Particularly accurate parameters could be obtained from semicrystalline fibers (16), but the same B form pattern was given by fibers with relatively little evidence of intermolecular interactions (17, 18). This B-form pattern corresponds closely to a 10.0-fold helix (P = 34 Å; p = 3.4 Å).

A number of previous studies suggest that the B form is present in solution. It is worth noting that, at high relative humidity, even in fibers the DNA may be highly hydrated [87 g of water per 100 g of lithium DNA at 92% relative humidity (16)]. A diffraction study by Neville and Davies (7) showed that the B form was present in even more highly hydrated fibers. As a control for diffraction studies of dye–DNA complexes, they collected patterns from DNA fibers that were exposed to a mist of water. The patterns of such wetted fibers were clearly of the B form. Over extents of water uptake that increased the spacing between molecules by as much as 2-fold, they found P to be invariant. Our values for P are also essentially invariant with water uptake. Neville and Davies gave no values for the p for their wetted DNA fibers, so that the number of residues per turn of the helix was not defined. Diffraction studies on unoriented gels of DNA also suggested the presence of a basically B-form structure. Wide-angle patterns obtained from such samples have been interpreted to be generally consistent with a B-type structure (19), whereas light-scattering and small-angle x-ray scattering results generally indicate a mass per unit length consistent with the B form (20).

The p of a regular polymer of known chain length may be obtained from a determination of the contour length of the molecule as carried out by electron microscopy. Vollenweider *et al.* (21) found by this approach that freeze-dried samples of DNA gave p = 3.43 ± 0.08 Å, in agreement with a B DNA structure. Samples treated in ways likely to give A or C forms or after intercalation with ethidium bromide gave p values consistent with the respective structures.

The results described up to this point are all consistent with a B form of DNA in DNA samples that are likely to be representative of DNA in solution. In contrast, several recent reports have suggested that distinctly different DNA structures may exist in solution. In a theoretical study, Levitt (4) has pointed out that packing forces in fibers would tend to favor integral helices (22) and therefore the B-form structure in fibers might relax to significantly different conformations when freed of intermolecular interactions. Starting with the B DNA coordinates, he allowed movements of the atoms in order to find lower energy structural arrangements of DNA that were still basically antiparallel base-paired double helices. We disagree with his conclusions on several grounds. Clearly, *both* helical parameters must be free to change in going from the B form to such pos-

tulated lower energy structures, yet in all his calculations Levitt fixed p at the fiber value and allowed only P to vary. The base-pair position and tilt relative to the helix axis are directly dependent on both parameters (23). For example, the tilt of a planar DNA base pair would be expected to more than double (from 6° to 14°) for a change in p from 3.3 Å to 3.5 Å. Hence, his considerations only extend to the subset of possible structures with p = 3.4 Å. It is perhaps this constraint that leads all of the "low-energy" conformations to be relatively unusual from a structural point of view. All of Levitt's models have greatly tilted bases with strong mutual twisting between the bases of a given base pair. Levitt's models also utilize a very unusual sugar conformation (C_1'-*exo*). Energy-minimization calculations were used to derive 9-, 10-, 11-, and 12-fold structures (p = 3.38 Å in all cases). By interpolating between the calculated energy levels of these models, he suggested that a 10.6-fold helix would be favored in solution. This value is not within experimental error of our estimate of 9.9 residues per turn in wetted DNA samples.

In a second paper raising the question of changes from the B DNA structure in solution, Griffith (5) presented electron microscope measurements of the length of DNA; in contrast the Vollenweider *et al.* (21), Griffith found p = 2.9 Å. Different techniques of sample preparation were used in the two studies, so it is difficult to assess the difference in results. In a third study, Hogan *et al.* (24) inferred a non-B-form geometry of DNA in solution, based on electric dichroism results. Highly tilted base-pairs were postulated, with considerable twist between the members of each pair. The conclusions of Hogan *et al.* are dependent upon certain assumptions about the direction of the transition moment and about the mechanism of orientation by electric fields. The validity of some of these assumptions has recently been questioned (25).

Wang (6) recently proposed that DNA in solution has 10.4 base pairs per turn. He measured the effect of oligonucleotide insertions of known length into closed circular DNA. The resulting changes in electrophoretic mobility were used to infer this value, based upon topological considerations. The basis for the difference between Wang's result and our own is not known; the great difference in DNA concentration (as well as counterion concentration) in the two experimental systems may be of significance.

Finally, the helical parameters of DNA in solution have implications for the supercoiling of DNA in nucleosomes (26). The change in linking number upon nucleosome formation must be reconciled with the helical parameters both of DNA in solution and as supercoiled nucleosomal DNA. At present, the reconciliation of these data is still speculative, given the uncertainties in the models for DNA in nucleosomes and in solution.

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