Curved DNA: Design, synthesis, and circularization

(DNA sequence periodicity/dinucleotide wedges/DNA anomalous electrophoresis/DNA circles)

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ABSTRACT Curved DNA molecules and unusually small circles have been obtained by ligation of synthetic 21-base precursors:

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\begin{align*}
5' & \quad \text{TCTCTAAAAATATATATATATATAT\ldots} \\
& \quad \text{TCTCTAAAAATATATATATAT\ldots} \\
& \quad \text{TCTCTAAAAATATATATATAT\ldots} \\
3' & \quad \text{TCTCTAAAAATATATATATAT\ldots}
\end{align*}
\]

The ligation resulted in the formation of double-stranded oligo-(precursor)s possessing a strong 10.5-base-pair (bp) periodicity of the runs of adenines. Two-dimensional polyacrylamide gel electrophoresis of the ligation products showed two distinct families of spots: (i) noncircular oligo(precursor)s of 21 to 231 bp (1- to 11-mers) and (ii) four circles from 105 to 168 bp (eluted and analyzed by denaturing gel electrophoresis). The noncircular oligomers exhibited anomalously slow migration, as if they were as much as three times longer than they actually are. The amount of circular products peaked sharply at 126 bp, near which size the circles have been estimated to be nonconstrained both torsionally and in terms of binding. The nonconstrained circularization provides a technique for the direct measurement of the inherent curvature of DNA in solution. From the size of the circles, an estimate of 8.7° is obtained for the absolute value of the AA-TT wedge angle (roll and tilt combined).

The "wedge" model of inherently curved DNA was suggested (1, 2) on the basis of a weak 10.5-base-pair (bp) periodicity observed in chromatin DNA sequences and is shown in Fig. 1. In this model each of the 10 stacks of adjacent bp (corresponding to the 10 possible dinucleotides) forms a wedge slightly deflecting the DNA axis in one direction or another. The word deflection here and below does not imply any deformation (bending) by force. Thus, the path of a DNA axis would generally look like a mixture of zigzag and unidirectionally curved lines, due to the accumulated small deflections. Two identical dinucleotides would deflect the DNA axis in the same direction, if placed in the DNA double helix at a distance which is approximately a multiple of the 10.5-bp pitch of natural DNA (3–6), see Fig. 1. Thus, DNA fragments possessing a statistical preference for the 10.5-bp periodicity of dinucleotides of the same wedge direction should be curved. Below we shall distinguish between such "curved" molecules—i.e., curvature due to their chemical conformation (sequence-dependent inherent curvature)—and "bent" molecules—i.e., curvature due to thermal motion or to the binding of proteins.

This rationale has been used for sequence-directed mapping of nucleosomes by computerized search for periodical stretches of DNA sequences (7). Of those several nucleosome binding sites, which have so far been identified experimentally with accuracy better than or about 5 bases, all happen to coincide exactly (±5 bases) with the computer mapped sites (7). The mapping of nucleosomes acquired an additional importance after it was found (8) that a shift of a nucleosome with respect to a promoter by only a few bases changes the gene expression rate in vitro by up to two orders of magnitude. There are also some indications that DNA curvature might play a role in transcription initiation in procaryotes (9, 10).

It has been found that some DNA fragments migrate anomalously slowly in nondenaturing polyacrylamide gels (refs. 11–17; ref. 10 and others, reviewed in ref. 18). That this effect was caused by the inherent curvature of the DNA fragments was first suggested by Marini et al. (11). The physical interpretation is that the more curved molecules encounter higher friction in the narrow pores of the polyacrylamide gel that slows their rate of migration.

Marini et al. (11) also found that the kinetoplast DNA fragment, exhibiting an especially large migration anomaly, has a very pronounced periodical distribution of AA (TT) dinucleotides. Periodical distributions of AAs and TTs were later found in all other anomalously migrating DNA fragments as well (18). The analysis of nucleosomal DNA sequences shows too that the 10.5-base periodicity is more pronounced for AA (TT) than for other dinucleotides (2, 7, 19). All of this, perhaps, suggests that the wedge angle between the base pairs in the minihelix AA-TT might be larger than in other dinucleotides (for review see ref. 18).

One interesting observation (17) illuminates the role of the sequence periodicity in the anomalous migration. A tandem 10-bp repeat containing a single AATT tetranucleotide was compared with a repeat of the same sequence shorter by 2 bp. The former was found to exhibit a migration anomaly, while the latter migrated normally. This result argues against any possible "scalar" explanation of the migration anomaly (e.g., due to anomalous flexibility).

The purpose of this work was to directly check whether a synthetic double-stranded DNA designed with a 10.5-bp periodicity of oligo(dA) runs can curve enough to form small mini-circles, and if so, to determine the wedge angle of the AA-TT duplex.

MATERIALS AND METHODS

Design. The two 21-base strands of the precursor

\[
\begin{align*}
5' & \quad \text{TCTCTAAAAATATATATATATATAT\ldots} \\
& \quad \text{TCTCTAAAAATATATATATATAT\ldots} \\
3' & \quad \text{TCTCTAAAAATATATATATATAT\ldots}
\end{align*}
\]

were designed in such a way, that: (i) none of the two strands would fold back into hairpins; (ii) the sticky ends would be sufficiently cohesive; (iii) the DNA helical pitch in solution (helical turn of 360°) would exactly equal 10.5 bp (half of the sequence repeat). This is important if the ligated circles are to be torsionally relaxed. All helical twist angles for individual combinations of stacked base pairs were taken from

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Abbreviations: 2-D, two dimensional; bp, base pair(s).
Kabsch et al. (20); and (iv) the DNA curvature would not depend on whether tilt or roll wedge happens to be dominant in the AA·TT duplex. This was one of the reasons why in the designed precursors the stretches between the oligo(dA) runs were not filled with oligo(dT) runs and vice versa.

Synthesis, Labeling, and Ligation. The two DNA strands, shown above, were synthesized, and their 5' ends were radioactively labeled with 32P essentially as described (21, 22). Ligations with 400 units of T4 DNA ligase (Amersham) were carried out at 12°C for 16 hr in 50 mM Tris-HCl (pH 7.6), 10 mM MgCl2, 1 mM ATP, 10 mM dithiothreitol. The DNA concentration was 0.6 mg/μl (0.1 μM of 5'-OH ends).

Analysis of DNA Fragments by Gel Electrophoresis. The ratio of acrylamide to bisacrylamide was 19:1 (wt/wt). All the gels were brought to 0.1 M Tris-borate (pH 8.3), 2.5 mM EDTA (TBE buffer). For denaturing gels urea was added to a final concentration of 7 M. The gels were run at room temperature in TBE buffer at 10 V/cm for non-denaturing gels and 30–40 V/cm for denaturing gels. The gels were exposed to preflashed x-ray films (Agfa, Belgium). The x-ray films were optically scanned at 500 nm with a DU-8 Beckman spectrophotometer.

Two-Dimensional Gel Electrophoresis. In the first dimension the DNA was loaded on a 4% polyacrylamide disc gel prepared in a 16-cm long, 1-ml disposable pipette (3 mm wide) and electrophoresed as described above until the bromophenol blue marker had migrated 11.5 cm. The gel was exposed to an x-ray film at 4°C for several hours, and the region containing practically all the DNA fragments obtained (14.5 cm) was laid on top of a 1.4-mm thick and 35 cm long slab gel. Acrylamide (10%) containing 50 μg of chloroquine phosphate/ml (Teva, Jerusalem, Israel) was allowed to polymerize around the disc gel. The electrophoresis buffer was TBE containing 50 μg of chloroquine phosphate/ml that was constantly recirculated between the electrode compartments. The gel was exposed to an x-ray film at room temperature (to prevent formation of crystals).

RESULTS AND DISCUSSION

Ligation of the synthetic 21-base precursors

\[
\begin{align*}
5' & \quad TCTCTTACATATATAAAAA \\
& \quad TTTTTATATTTTATAGAGA \\
3' & \quad TCTCTTACATATATAAAAA
\end{align*}
\]

resulted in the formation of oligo(precursor) possessing the strongest possible 10.5-bp periodicity of the runs of adenines (thymines). The main question was whether this periodicity would yield a sufficient inherent curvature to produce DNA circles of unusually small size.

To find out whether there were any circles among the ligation products, the latter were subjected to a two-dimensional (2-D) polyacrylamide gel electrophoresis (Fig. 2), in which the second dimension was run in the presence of an intercalating agent (chloroquine). The intercalating agent should distort the presumed C-shaped (noncircular) molecules differently from the covalently closed O-shaped ones (circular). Indeed, two separate families of spots are observed on Fig. 2, as expected. An a priori physical rationale for running such 2-D gels was that the open ends in the C-shaped (as opposed to the O-shaped) molecules should allow the intercalating agent to increase the electrophoretic mobility by reducing the overall DNA curvature through twisting the otherwise uniformly curved DNA (the lower family of spots in Fig. 2).

To confirm the nature of the two families of spots and also to measure the size of the presumed circles, spots a, b, and c in Fig. 2 were extracted from the gel and electrophoresed in denaturing conditions in separate lanes (denoted as a, b, and c, respectively, in Fig. 3A). In contrast to noncircular molecules, denatured DNA originated from double-stranded circles display three bands due to occasional nicks. The main band contains denatured covalently closed double-stranded circles (two interwound single-stranded circles). The two additional bands, both resulting from a nick in the double-stranded circle, contain single-stranded linear and circular molecules of the same size. The nicks might occur for three possible reasons: (i) radioactive decay of 32P; (ii) incomplete kination (leading to the absence of a 5' phosphate); or (iii) incomplete ligation in one of the two strands. In the resulting gel (Fig. 3A) each of the a and b lanes does show three bands. The fastest of the three bands (linear molecules) comigrates with one of the linear oligomers (multiples) of the 21-base precursor. As expected, the single-stranded circle migrates slower than its linear counterpart (e.g., ref. 23).

Fig. 3B shows an optical density scan of lane b of Fig. 3A, while Fig. 3C shows a scan of the same material electrophoresed 10 days later. The number above each peak indicates its relative area (% of total). A comparison of Fig. 3B with Fig. 3C shows that after 10 days of radioactive decay, an appreciable portion of the main band (two interwound circles) was found in two faster bands (single circle and linear molecule) and some of the material from the second band (single circle) went into the third band (linear molecules).

Thus, the actual sizes of the circular molecules a and b in Fig. 2 are determined to be 105 and 126 bp (5- and 6-mers of the precursor) from the migration of the fastest bands (linear fragments) in lanes a and b of Fig. 3A. Lane c of Fig. 3A
The intercalation of chloroquine into the DNA molecule introduces a slight difference in the migration of the nicked and covalently closed molecules. The latter gel the circular and linear natures of the two families as well as the sizes of the molecules were again confirmed on a denaturing gel. In yet another 2-D gel a similar family separation (though of a different physical nature) was also observed with the second dimension electrophoresed in denaturing conditions (not shown).

An interesting feature of Fig. 2 is the doublet structure of the spots of the upper family. This effect has a natural explanation. In the second dimension the intercalating agent should introduce a different torsional deformation into the nicked circles as compared to the covalently closed ones resulting in a slight difference in migration. In the first dimension of Fig. 2 the nicked and covalently closed circles of each doublet comigrate. The comigration could be expected, since the closed circles were designed to be torsionally unconstrained in the absence of intercalating agent. The first dimensional comigration is a valid evidence of the lack of torsional constraint, only in view of the intrinsic test in the second dimension, where the torsional deformation noticeably changed the mobility of the small circles.
The separation of the two families of spots on the 2-D gels made it possible to measure the migration anomaly of the noncircular molecules on 4% and 8% polyacrylamide/nondenaturing one-dimensional gels (Fig. 4). The ratio of the apparent to actual lengths of the DNA molecules reaches 3 for the 8% gel. This indicates that the noncircular molecules must have a substantial inherent curvature.

Imagine a DNA circle of such a size that it has the same curvature as its nonclosed, relaxed, C-shaped counterpart of the same sequence. Such a circle should be nonconstrained in terms of bending. We shall call the size of this circle "the nonconstrained circle size." How small this DNA is is worth discussing.

The intensities of the upper family of spots in Fig. 2 (circular molecules) indicate that the most abundant circularization occurred at 126 bp. The question is whether this value is larger, smaller, or close to the above nonconstrained circle size. The following are some reasons that lead us to believe that the latter alternative is probably the right one.

(i) Optimal size of circularization. The circularization efficiency is represented by the ratio of circular to noncircular ligation products of the same size, rather than by the amount of the circular product alone. Fig. 5 shows that this ratio, as a function of the size of the molecules, reaches its maximum at a strikingly small size of around 137 bp and then rapidly drops above 147 bp. In spite of the presence of noncircular products of up to 231 bp in the ligation mixture, no appreciable amount of circles larger than 168 bp is formed (see Fig. 2). This is in sharp contrast to the circularization of essentially noncurved DNA (24), where the fragments with the highest circularization efficiency were all 366 to 1361 bp, and the circularization efficiency dropped rapidly below 300 bp, being for a 126-bp fragment less than 1% of that for the 1361-bp reference fragment.

The location of the efficiency peak of circularization by thermal bending (24) depends on the DNA flexibility. The optimal size of circularization is proportional to the persistence length, the only physical parameter relevant to thermal bending of essentially straight fragments in constant conditions. In our DNA poly(A)-poly(T) constitutes 50% and alternating poly(AT)-poly(AT) 25% of the sequence. The persistence length of both was found to be smaller than that of random DNA—for poly(A)-poly(T) by 30–40 bp (25), and for alternating poly(AT)-poly(AT) by about 50% (26). With the persistence length of random DNA equal to 150 bp (27–29), our oligo(precursor) are expected to have the persistence length of 105 ± 15 bp. This 30% decrease in the persistence length would lower the optimal size of circularization by thermal bending in the same proportion, slightly shifting the broad plateau of 366 to 1361 bp (24), which is not enough to match the sharp peak found at 137 bp in Fig. 5.

(ii) Width of circularization efficiency peak. The curve in Fig. 5 crosses the level of 1/e of the maximum at 105 and 173 bp, a difference of only 65% (Fig. 5). Shore et al. (24) found that at the same level of 1/e of the maximum, the fragments differed by more than 1000 bp in length, and the relative size difference was almost one order of magnitude. This is another indication that the nature of the peak shown in Fig. 5 is different from that reported by Shore et al. (24). Thus, bending by thermal motion of essentially straight DNA explains neither position nor width of the circularization efficiency peak shown in Fig. 5.

(iii) Interpretation by means of inherent curvature. Suppose the nonconstrained circle size L corresponds to the middle of the curve shown in Fig. 5 so that L = 137 bp, while the curve itself describes the Boltzmann distribution of bending constraint energy. That is, the left and the right slopes of the peak represent overbent and underbent circles, respectively. The KT energy of bending constraint corresponds to the half-width of the peak at 1/e of the maximum. Since the persistence length P of our DNA was estimated above as P = 105 ± 15 bp, one obtains the expected half-width, W, as

\[ W = \frac{L}{\pi} \sqrt{\frac{L}{2P}} = 35 \pm 2 \text{ bp} \]

(on thermal deformation of DNA see, e.g., ref. 30), which is remarkably close to the observed value of 34 ± 3 bp (Fig. 5).

However conclusive this result might seem, one should ask whether the assumed 137 bp nonconstrained circle size is realistic. So far, there have been two estimates for the AA-TT wedge angle in solution: 5° (31) and 11° (32). For our sequence these estimates would result in nonconstrained circle sizes of 240 bp and 110 bp, respectively, thus making the value of 137 bp estimated from Fig. 5 not unreasonable.
Therefore, unlike the thermal bending, the inherent curvature explains quantitatively both the position and the width of the circularization efficiency peak, with all free parameters (peristalsis length and AA-TT wedge angle) estimated in earlier experiments.

Should the average circles obtained here be constrained in terms of bending, the optimal size of circularization would depend on the DNA concentration in the ligation mixture. Thus, a parallel ligation was carried out with the precursor concentration 10 times higher than that used for Fig. 2. The sizes of circles obtained were the same within the accuracy of 20% (not shown).

Assuming the dinucleotide wedge hypothesis of the DNA curvature, an estimate of the absolute value of the AA-TT wedge angle (roll and tilt combined) is found from the circularization optimum derived from data in Fig. 5 to equal 8.7°. This value has been obtained by, first, computing the AA-TT wedge that would produce the circular 6-mer of the precursor (126-bp circle in Fig. 6A). The angle obtained (9.5°) had, then, to be corrected to for the actual optimum length from Fig. 5, about 137 bp (a nonmultiple of 21 bp), resulting in the value of 8.7°. The contribution of dinucleotides other than AA-TT to our DNA curvature is small, in view of the large number of AA-TTs and the 10.5-base spacing of the oligo(A) runs. This is correct even if some dinucleotide wedge angles are comparable to that of AA-TT. Unlike AA-TTs, the contribution of each of the other dinucleotides to the total curvature is equally probable to be positive or negative. These unknown contributions introduce an uncertainty of 14° to 2° into the estimate of 8.7° for the AA-TT wedge angle. Fig. 6B shows a computer graphed constrained C-shaped 5-mer of the 21-base precursor, where each AA-TT wedge has the angle of 8.7°.

An alternative model of DNA curvature (31, 33) suggests that due to a different DNA conformation of oligo(dA) oligo(dT) runs, the DNA axis suffers some deflection at each of the two junctions of such a run with the rest of the DNA molecule. If, indeed, such a junction deflection is responsible for the overall curvature, its angle can be similarly estimated for the above circularization optimum of 137 bp in Fig. 5 to be around 14°. This model was applied to the kinetoplast DNA (31) together with the dinucleotide wedge model, and the two models were found to be indistinguishable for that fragment. The same is true for the circles described above. The difference between the two models can be reduced to the question of whether an isolated AA-TT flanked by different base pairs possesses a smaller wedge angle than an AA-TT within an oligo(dA) run, due to a different conformation of oligo(dA).

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