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Bent helical structure in kinetoplast DNA
(DNA conformation/sequence periodicity/trypanosomatid/mitochondrial DNA)

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ABSTRACT We have investigated the unusual physical properties of a restriction fragment of Leishmania tarentolae kinetoplast DNA. A gel-purified fragment comprising slightly more than half of a minicircle was determined by Maxam–Gilbert sequence determination to be 490 base pairs (bp) in length. This fragment has dramatically anomalous electrophoretic behavior; it has an apparent size of 450 bp on a 1% agarose gel but migrates as 1,380 bp on a 12% polyacrylamide gel. However, in gel filtration on Sephacryl S-500, the fragment elutes with an apparent size of 375 bp. Finally, it behaves anomalously in electric dichroism experiments. Field-free rotational relaxation times from transient electric dichroism studies are highly sensitive to effective molecular dimensions. The rotational relaxation time of the kinetoplast fragment is smaller than that of a 300-bp control fragment from pBR322. Because rigorous control experiments rule out the possibility that this fragment is modified, these anomalous properties must be dictated by the sequence itself. Fragment behavior indicates that it has an unusually compact configuration; we propose that this molecule contains a region of systematically bent B-DNA. This model accounts for the fragment's difficulty in snaking through the pores of a polyacrylamide gel, its ease in diffusing into Sephacryl beads, and its smaller rotational relaxation time. Bending of this molecule may be caused by periodicities in the DNA sequence.

The past few years have seen a revival of interest in DNA helical structure. This renewed interest goes beyond the solution of the intricacies of crystallized synthetic oligonucleotides even as it reaffirms their worth. It represents a growing conviction that DNA is not just a monotonous repository of coded genetic information. Rather, it has considerable conformational flexibility, which is likely to be significant for the interactions of DNA with protein, RNA, and other DNA. As alternatives to the well-known B form of the DNA helix, considerable attention has been focused on the A form and on left-handed Z-DNA. There also has been much interest in the possibility of cruciforms, kinks, and bends in the DNA helix. (For reviews, see refs. 1–3.)

We have found that a restriction fragment of kinetoplast DNA from Leishmania tarentolae has unusual physical properties that are best explained by postulating an unusual helical conformation. Kinetoplast DNA is the mitochondrial DNA of parasitic protozoa of the family Trypanosomatidae (see refs. 4 and 5 for review). Kinetoplast DNA is organized into networks of thousands of interlocked DNA circles. Each cell has one network within its single mitochondrion. A network contains a few large maxicircles, which are similar in function to the mitochondrial DNAs of other eukaryotic species. However, most of the network consists of thousands of minicircles; their function is not yet known. It is highly unlikely that minicircles have a coding function, as their sequences are riddled with stop codons and they do not appear to be transcribed. Minicircles vary in size from species to species, and in L. tarentolae they contain about 800 base pairs (bp). Furthermore, minicircles also vary in sequence in different species; there is even considerable sequence heterogeneity within a single network. L. tarentolae minicircles have three major sequence classes and several minor ones.

In this paper, we describe some unusual physical properties of a restriction fragment from an L. tarentolae minicircle. The behavior of the fragment in gel electrophoresis, gel filtration, and electric dichroism experiments indicates that the molecule has an unusually compact conformation; it seems likely that the molecule contains a region of systematically bent B-DNA helix. Because control experiments rule out the possibility of modification of the molecule, it must ultimately be the sequence itself that causes the fragment to bend. This bending may be due to sequence periodicities.

MATERIALS AND METHODS

The kinetoplast fragment used in these experiments is Mbo I fragment A from class II minicircles of L. tarentolae (6). Its isolation, cloning in pBR322 as pFE103, isolation from the plasmid, and sequence determination will be described elsewhere. The fragment derived from L. tarentolae cells was used in the gel electrophoresis shown in Fig. 2. The cloned fragment had identical anomalous electrophoretic behavior; it was used for gel filtration and electric dichroism experiments.

The electric dichroism apparatus is a modified version of the one described earlier (7). Low-field dichroism experiments are carried out by applying a rectangular exciting pulse of alternating polarity from a Cober 605P pulse generator across the dichroism cell. The cell is made of clear Lexan with sapphire windows and a pair of platinum electrodes. The electrode gap is 5 mm. Data acquisition and processing are done with a LeCroy 2256 waveform digitizer interfaced to a Digital Equipment Corporation PDP-11/40 computer. The instrument operates in the signal-averaging mode; the computer collects and averages each data set up to a predetermined number of sweeps. An interactive computer program is used to analyze the dichroism data and determine the best fit of the decay portion of the signal to a single exponential.

Restriction fragments suitable for the electric dichroism studies were prepared from pFE103. The final step in restriction fragment preparations was an electrophoretic separation on a 5% polyacrylamide gel that had been run for 48 hr with several changes of buffer prior to the experiment. If the gel is not prepared in this way, the DNA isolated from the gel has drastically altered electrical properties, possibly due to binding to the DNA of contaminating macromolecules in the gel. DNA fragments were electroeluted from the gel onto DE 81 paper in a Bio-Rad
Trans-Blot apparatus (8). They were eluted from DE 81 paper, rebound to a DE 52 cellulose column (5 mm × 10 mm), washed extensively with 10 mM Tris-HCl, pH 8/1 mM EDTA, and eluted with 1 ml of 10 mM Tris-HCl, pH 8/1 mM EDTA/1 M NaCl. DNA concentrations were determined with a Cary 219 spectrophotometer.

The computer program used to calculate the positional autocorrelation functions was written in FORTRAN-77 and run on a VAX-11/750 computer in the Department of Chemistry, Yale University. Other computer analyses were done with the Stanford MOLGEN Project and the National Institutes of Health SUMEX-AIM Facility.

RESULTS

The Actual Size of the Kinetoplast Fragment Is 490 bp. In a study (6) aimed at the mapping of L. tarentolae minicircle restriction sites, we made the unexpected discovery that some fragments electrophorese extremely slowly, relative to their size, on polyacrylamide gels. Similar findings also have been made on other L. tarentolae minicircle fragments (9, 10). We selected the Mbo I fragment A of class II minicircles for further study because it is easily purified. Fig. 1 shows its nucleotide sequence. It is 490 bp in length and only slightly (53%) A+T-rich.

The most striking aspect of the primary sequence is the presence of alternating G+C- and A+T-rich runs (>70% G+C or A+T), ranging from 10–40 nucleotides in length. Within the A+T-rich runs of the strand shown in Fig. 1, there are runs of As (4–8 bases each) and a few runs of Ts (4 or 5 bases each).

A computer search for inverted repeats revealed no possible cruciforms with short loops and perfectly paired stems. Those structures that could be drawn were energetically unfavorable because of considerable mismatch in the stem or large loop size. Furthermore, S1 and mung bean nuclelease digestions on both isolated fragment and fragment inserted in supercoiled pBR322 revealed no single-stranded regions (unpublished data).

The Kinetoplast Restriction Fragment Migrates Anomalously on Polyacrylamide Gels. Electrophoresis on either agarose or polyacrylamide gels has become the standard method for rapid estimation of the size of DNA fragments. Exact determination of fragment length by nucleotide sequence determination has verified the accuracy of estimates based on electrophoretic migration.

The dramatically anomalous behavior of the 490-bp kinetoplast fragment in gel electrophoresis is shown in Fig. 2. Compared to a φX174 Hae III marker digest, the kinetoplast fragment migrated on polyacrylamide gels as if its size were 1,380 bp on 12% gels, 1,240 bp on 8% gels, 1,040 bp on 6% gels, 610 bp on 4% gels, and 490 bp on 2% polyacrylamide/0.5% agarose gels. On 1% or 2% agarose gels, it had an apparent size of 450 bp. Discrepancies of 10–20% between the size estimates on agarose and polyacrylamide gels have been noted before (11, 12). However, to our knowledge, no fragment from any other source has been shown to behave in polyacrylamide gels as though it were >2.5 times its actual size.

Fig. 2B documents the trend of anomalous behavior in polyacrylamide gels. The degree of anomaly depended on the porosity of the gel; the apparent size of the kinetoplast fragment approached the actual size as the percentage of polyacrylamide in the gel decreased.

The Kinetoplast Fragment Behaves Anomalously on Gel Filtration. Gel filtration has been less widely used to measure the size of DNA fragments. However, it should provide an accurate estimate of the number of base pairs in DNA fragments because the length of fragments would be expected to dominate their partition between gel beads and the mobile phase.

To test the gel filtration properties of the 490-bp kinetoplast fragment, it was mixed with φX174 Hae III control fragments and was filtered through a column of Sephacyr S-500. The DNA fragments present in each fraction of eluate were electrophoresed on an 8% polyacrylamide gel. Fig. 3 shows the autoradiograms of these gels.

The control fragments behaved in the expected manner for molecules undergoing separation by gel filtration. They were eluted from the column at volumes that were inversely proportional to the logarithm of their size (Fig. 3 Inset). The larger control fragments were several times the persistence length of DNA and must have a considerable degree of flexibility. Nevertheless, diffusion into the stationary phase did seem to be linearly related to the longest dimension of the restriction fragments.

However, the kinetoplast fragment did not elute as expected of a fragment of either its actual size or its apparent size on polyacrylamide gels. It eluted as though it were 375 bp in length.

The Kinetoplast Fragment Behaves Anomalously in Electric Dichroism Experiments. Transient electric dichroism is a sensitive method for studying the rotational dynamics of DNA molecules in solution. This method exploits the electrical and optical anisotropy of DNA. An electric field is applied to the sample, and the absorption of polarized light is observed. Because the DNA molecules are electrically anisotropic, they orient in the field; because they are also optically anisotropic, there is a change in the absorption of polarized light. Eventually a steady state is reached, and the optical properties do not change with time as long as the field is constant. Then, when the field is abruptly removed, the field-free decay of optical anisotropy may be observed. The characteristic time for this

![FIG. 1. Nucleotide sequence of the 490-bp restriction fragment of L. tarentolae class II minicircle. Sequence determination strategy will be described elsewhere. The strand shown has the 4-bp Mbo I recognition site at each end. Nucleotide composition of the strand shown is adenine 28.8%, cytosine 22.6%, guanine 24.3%, and thymine 24.3%.](image-url)
FIG. 2. Gel electrophoresis of the kinetoplast fragment. (A) Comparison of the mobilities of the kinetoplast fragment (lanes K) and Hae III fragments of φX174 (lanes M) on agarose and polyacrylamide (PA) gels. For polyacrylamide gels, the φX174 DNA was uniformly labeled with \(^{32}\)P phosphate, and the kinetoplast fragment was 5' end-labeled. The agarose gel (Sigma, low electroendosmosis) was run horizontally at 2 V/cm for 16 hr in 90 mM Tris borate, pH 8.3/2.5 mM EDTA. The polyacrylamide gels (40 cm) were run vertically at 3 V/cm in 40 mM Tris-HCl/20 mM sodium acetate/2 mM EDTA, pH 7.8, until the bromphenol blue marker reached 23 cm (15-18 hr). For 6-12% gels, a 1:40 N,N'-methylenebisacrylamide/acrylamide ratio was used; for 2% and 4% gels, the ratio was 1:20. The 2% polyacrylamide gel was reinforced with 0.5% agarose. Gels were analyzed by autoradiography (polyacrylamide) or ethidium staining (agarose). (B) Plot of gel migration of anomalous fragments as a function of the percentage of polyacrylamide. Bromphenol blue (BPB) marker was electrophoresed to 23 cm, and the migration distance of each fragment was measured from autoradiograms. Migration distances of φX174 Hae III marker fragments of indicated nucleotide sizes (light line), the kinetoplast fragment (heavy line), and a hypothetical "normal" 490-bp fragment (-----) are indicated. Discontinuity in the migration of control fragments between 6% and 4% polyacrylamide is due to the increased bisacrylamide in lower percentage gels.

The rotational relaxation time is the rotational relaxation time, \(\tau_r\), which is related to the rotational diffusion constant of the molecule, \(D_r\), by the equation \(\tau_r = 1/6 D_r\) (13). For dilute solutions of macromolecules, \(D_r\) is a measure of the frictional resistance of the molecule to rotation and, thus, is related to the size and shape of the molecule. In the case of short, rod-like DNA molecules (14), \(D_r\) is inversely proportional to the length cubed (bp)\(^3\). For longer molecules, where there are significant departures from rod-like behavior, a more complicated relationship has been found (15).

Table 1 shows the rotational relaxation times for single exponential decay of a series of Hpa II restriction fragments of pBR322. These results are in reasonable agreement with values measured in earlier studies (16, 17). For the Hpa II fragments of the size range measured here, \(\tau_r\) increased with fragment length proportional to \(\text{bp}^{1.4}\).

The rotational relaxation time of the 490-bp kinetoplast fragment was 9.3 \(\mu\)sec, smaller than even the 309-bp control fragment.

**DISCUSSION**

The 490-bp *L. tarentolae* kinetoplast fragment has three unusual physical properties. First, relative to control fragments, it electrophoreses unexpectedly slowly on polyacrylamide gels. The anomalous migration rate approaches normal as the pore size of the gel increases. To our knowledge, no fragment has been reported to behave as anomalously in gel electrophoresis as this one. Second, when compared to the same control fragments on a gel filtration column, the kinetoplast fragment behaves as though it were smaller than its actual size. It elutes at a position expected for a molecule of 375 bp. Third, in electric dichroism experiments, the rate of rotational diffusion is faster than that of a 309-bp control fragment.

As will be described elsewhere, rigorous control experiments including deproteinization, RNase treatment, cloning in a wild-type or a nonmethylating *Escherichia coli* strain, velocity sedimentation, denaturation-renaturation, and treatment with single strand-specific nuclease rule out the possibility that this fragment is modified or has significant secondary structure (unpublished data). Therefore, these unusual properties must ultimately be a consequence of the sequence itself.

The anomalous physical properties of the fragment imply that it has an unusually compact structure, with its ends closer together than expected for its contour length. A compact conformation explains the fragment's anomalous behavior in gel filtration and electric dichroism experiments. Compared to normal control fragments, it would diffuse more readily into Sephacryl beads and would present less frictional resistance to rotational diffusion. It is less obvious why a more compact configuration should lead to reduced electrophoretic mobility in highly crosslinked polyacrylamide gels. Because the diameter of the average pore in a polyacrylamide gel is of the order of the diameter of the double helix (18), the electrophoretic mobility of DNA fragments in these gels may be sensitive to the dimensions of segments of the DNA which snake through the gel. A bent or curved DNA molecule would present a larger effective
cross section to the pores of the gel than would a normal molecule of the same contour length. Increasing the average pore size by decreasing the acrylamide concentration should reduce the sensitivity to effective helix cross section, in agreement with the fragment's nearly normal electrophoretic mobility in dilute gels.

Table 1. Rotational relaxation times of kinetoplast fragment and control fragments

<table>
<thead>
<tr>
<th>Fragments</th>
<th>Length, bp</th>
<th>$\tau_*$, $\mu$s</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hpa II</em> fragments of pBR322</td>
<td>309</td>
<td>16.6 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>403</td>
<td>23.6 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>527</td>
<td>33.3 ± 0.6*</td>
</tr>
<tr>
<td>Kinoplast</td>
<td>490</td>
<td>9.3 ± 0.6</td>
</tr>
</tbody>
</table>

*This value is the average of three or four measurements made at fields between 1.0 and 2.0 kV/cm. Dichroism measurements were made at 265 nm; the samples were at 6°C in 2 mM ionic strength buffer (0.3 mM NaH₂PO₄/0.6 mM Na₂HPO₄).

*The 527-bp fragment also had a second slow phase ($45 \mu$s) relaxation; this complex relaxation may be the result of fragment flexibility.

Because cruciforms or other secondary structures were not detectable, the best alternative model for a compact formation is that of a curvature in the molecule. We see two possible types of bent helix. The first is a composite helix with a bend at the junction of two helical types. For example, there may be a bend of about 26° at the junction of B- and A-form helices (19). However, circular dichroism studies show only a B-form helix in the L. tarentolae fragment (unpublished data). The second possible type of bent helix is a B-form helix that is systematically bent because of periodicities in its sequence. Trifonov and Sussman (20, 21) have suggested that certain combinations of adjacent base pairs may normally be slightly nonparallel because of local structural constraints. For example, potential energy calculations have predicted that in the duplex ApA/TpT there is a slight bend, with the wedge pointing toward the strand containing ApA (22). Alternatively, the molecule may be bending preferentially towards the major groove as found in a crystallized oligonucleotide (23). If such bends occurred randomly along a helix, they would cancel out. The periodic positioning of a bend once per helical turn would cause the molecule to curve systematically in a plane; occurrence of a bend at some other periodicity would introduce a writh into

**Fig. 3.** Sepharcl S-500 gel filtration of the kinetoplast fragment. Aliquots of a dX174 Hae III digest and the 480-bp kinetoplast fragment, both 5' end-labeled with polynucleotide kinase and [$\gamma$-32P]ATP, were mixed and loaded onto a 120 x 0.9 cm Sepharcl S-500 (Pharmacia) column equilibrated with 20 mM Tris-HCl, pH 8/1 mM EDTA. The sample was loaded in 0.5 ml of column buffer. The column was run at 12 ml/hr at room temperature, and 2-ml fractions were collected. Carrier tRNA (0.05 $\mu$g) was added to fractions containing radioactivity. They were ethanol precipitated, resuspended in 20 $\mu$L of buffer, and electrophoresed on 8% polyacrylamide gels. Standard lanes (Stds) show migration of dX174 Hae III fragments and the anomalous kinetoplast fragment (lane A). The large spot at the bottom of the dX174 lane is unincorporated [$\gamma$-32P]ATP. The smaller Sau3A fragments in lane A are derived from the pBR322 portion of a 766-bp BamHI/SalI fragment from which the kinetoplast fragment was released by Sau3A digestion. Numbered lanes contain the precipitated fragments from the corresponding Sepharcl fractions. The kinetoplast fragment is indicated (-). (Inset) Graph shows the relationship of elution volume to length of reference fragments (>) and kinetoplast fragment (\(\Delta\)).
the probability that a given subsequence of length \( m \), such as ApA, repeats itself a distance \( j \) bases away. Specifically,

\[
G_j = \left( \sum_{i=1}^{L-m+1} \delta_i \delta_{i+j} \right) / \sum_{i=1}^{L-m+1} \delta_i,
\]

where \( \delta_i \) is an index whose value is 1 when the particular subsequence starts at base \( i \) in a sequence of total length \( L \) and 0 otherwise. According to Eq. 1, the autocorrelation function is 1 when \( j = 0 \) (\( G_0 \) = 1). In an infinitely long random sequence, \( G_j \) should be a constant when \( j \) is larger than the subsequence length because the subsequences at \( i \) and \( i + j \) are uncorrelated.

free of protein. Because the periodicities of the kinetoplast fragment are imperfect, its curvature would be expected to be more complex and irregular.

We recently have found anomalous electrophoretic behavior similar to that of the *Leishmania* kinetoplast fragment in some minicircle restriction fragments from several other trypanosomatid species (unpublished observations). It is likely that a systematically bent helix is also the cause of their anomalous behavior. These studies suggest that it may be this particular structure rather than a particular sequence that is the conserved feature of kinetoplast minicircles.

We do not know the function of the natural curvature of kinetoplast DNA sequences. One possibility is that it facilitates binding of some protein to the bent region. Another possibility is that it facilitates condensation of the kinetoplast DNA network into the highly-organized disk-like structure present within the cell's mitochondrion (24).

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