

dependence between the two enzymes was found. Disulphide isomerase catalyses the reactivation of reduced proteins, and prolyl isomerase can accelerate the refolding of proteins with incorrect proline isomers. Our results do not support the suggestion⁹ that disulphide isomerase increases the rate of protein folding by catalysis of proline isomerization, but they agree with existing experimental evidence and mechanistic models that assume disulphide isomerase acts by facilitating the formation of correct disulphide bonds during protein folding *in vitro* as well as *in vivo*^{5,6,13}

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Structural details of an adenine tract that does not cause DNA to bend

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Runs of adenines (adenine tracts) have been implicated as the main determinant of sequence-directed DNA bending¹⁻⁴. The most widely used experimental test for bending relies on the observation that bent DNA migrates more slowly than straight DNA on a polyacrylamide electrophoresis gel¹⁻⁵. It was shown recently that the polymer (GTTTTAAAAC)_n runs with normal mobility on a gel, whereas (GAAAATTTTC)_n runs more slowly and thus appears to be strongly bent⁶. The observation that these similar sequences, which differ only in the order of the adenine and thymine tracts, adopt such different shapes offers a stringent test of theories to explain DNA bending. Although the wedge model for DNA bending has recently been elaborated⁷ to explain the gel mobilities of these molecules, we wished to determine experimentally the structural basis for the difference in bending. We report here measurements of the frequency of cleavage by the hydroxyl radical at each nucleotide of cloned versions of the two polymers (see Fig. 1). We show that the TTTTAAAA sequence does not display the cleavage pattern that is associated with bent DNA⁸, whereas the AAAATTTT sequence does. The observed sequence dependence of the cleavage pattern of an adenine tract is at odds with current models^{4,7} for DNA bending, which assume that adenine tracts always adopt the same conformation.

The hydroxyl radical, which we generate by reaction of the EDTA complex of iron(II) with hydrogen peroxide, initiates cleavage of a DNA strand by abstracting a hydrogen atom from a deoxyribose in the DNA backbone⁹. The hydroxyl radical cleaves mixed-sequence DNA nearly equally at each backbone position⁹. Previously we showed⁸ that the hydroxyl-radical cleavage pattern of bent kinetoplast DNA was instead markedly sinusoidal in appearance, with the minima of the cutting pattern phased perfectly with the 3' ends of adenine tracts. The cleavage pattern of the opposite, thymine-rich, strand was similar in shape

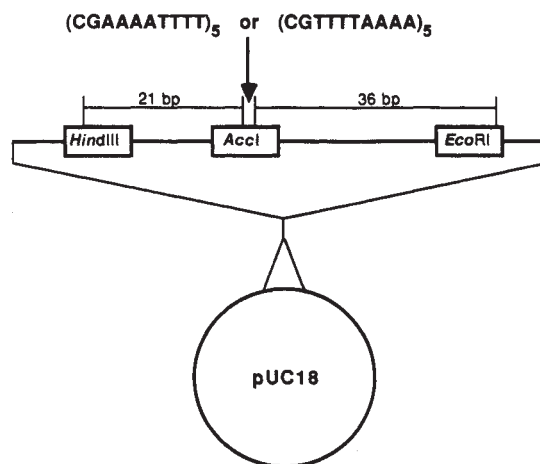


Fig. 1 Cloning oligomers of the sequences CGAAAATTTT and CGTTTTAAAA.

Methods. The partially self-complementary decanucleotides 5'-CGTTTTAAAA-3' and 5'-CGAAAATTTT-3' were synthesized by standard automated methods (Applied Biosystems) and purified by Mono-Q anion-exchange chromatography on a fast protein/peptide/polynucleotide liquid chromatography (FPLC) system. To clone an oligomer of one of the A-tract sequences, a decanucleotide duplex was ligated in the presence of *AccI*-cleaved pUC18. *Escherichia coli* (strain HB101) was transformed with the ligation mixture and plated on ampicillin plates²⁰. Clones containing plasmid with five repeats of a decamer were selected.

but shifted by one to three nucleotides in the 3' direction, indicating that the cleavage rate is correlated for nucleotides directly across the minor groove from one another. Our interpretation of the kinetoplast DNA cutting pattern therefore implicated a progressive narrowing of the minor groove from 5' to 3' along an adenine tract as a structural feature linked with DNA bending⁸. Oligo(dA)-oligo(dT) tracts have been shown by recent single crystal X-ray diffraction experiments^{10,11} to have a more narrow minor groove than mixed-sequence DNA.

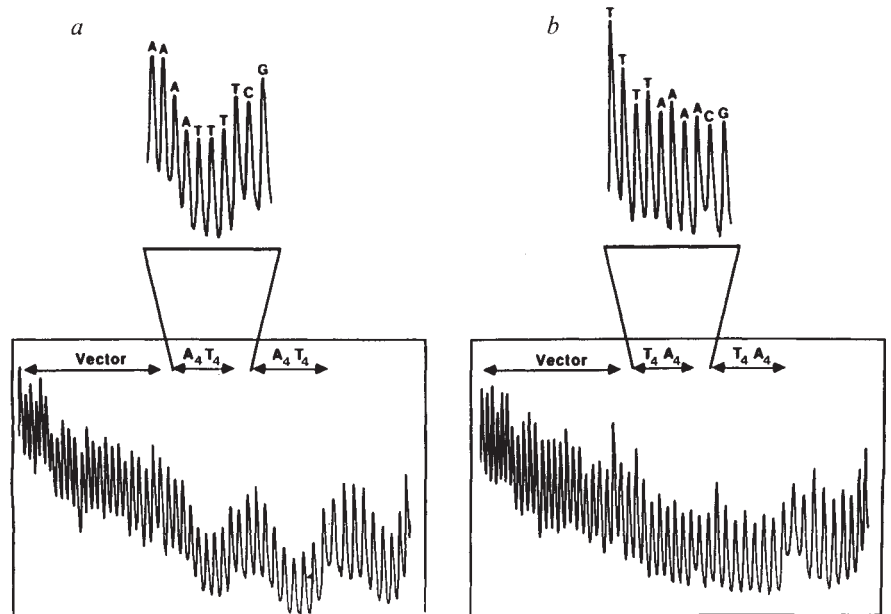
In Fig. 2 we compare the hydroxyl-radical cutting patterns of oligomers of the sequences CGAAAATTTT and CGTTTTAAAA. An important feature of the design of the experiment is that the restriction fragments studied contain mixed-sequence DNA from the cloning vector flanking the adenine tract oligomers. This internal control allows us to compare the cutting frequencies of an adenine tract and 'normal' DNA. We find that each repeat of a particular adenine-tract oligomer has the same cutting pattern, illustrating the reproducibility and sensitivity of the method.

The CGAAAATTTT sequence shows a clear sinusoidal cutting pattern, whereas the adjacent vector DNA is cut with a fairly even frequency that is higher than the minima in the CGAAAATTTT tracts (Fig. 2a). In contrast, the CGTTTTAAAA sequence (Fig. 2b) exhibits an almost even cutting pattern, similar in intensity to that of the flanking mixed-sequence DNA. We conclude that the adenine tracts in (CGTTTTAAAA)_n adopt a different conformation from the adenine tracts in (CGAAAATTTT)_n.

What are the different structures of the two sequences? Our interpretation of the cleavage patterns is that the minor groove of the AAAATTTT sequence periodically opens and closes in width, like bent kinetoplast DNA⁸, and that the TTTTAAAA sequence has a minor groove as wide as in mixed-sequence DNA. Several lines of evidence support these conclusions. The crystal structure of the dodecamer CGCGAATTTCGCG shows that the minor groove in the AATT sequence is narrower than in the CG regions¹². Two of the structural characteristics associ-

Fig. 2 Densitometer scans of autoradiographs showing the hydroxyl-radical cleavage patterns of the T_4A_4 and A_4T_4 oligomers. Arrows above the densitometer scans indicate oligomer and vector sequences. Two of the five repeats of each adenine-tract sequence are shown. A scan at expanded scale of one repeat of each sequence is shown at the top, with the sequence marked. *a*, The cleavage pattern of the A_4T_4 oligomer is sinusoidal, reminiscent of the cleavage pattern of kinetoplast DNA⁸. The cutting frequency decreases from the 5' adenine to the 5' thymine, and then increases. The cleavage pattern on the other strand (not shown) is nearly identical in shape but offset by 2–3 nucleotides to the 3' direction⁸, indicating that the observed changes in cleavage frequency are associated with structural features of the minor groove¹⁸. *b*, The T_4A_4 oligomer has a relatively even cleavage pattern, not much different from that of flanking vector DNA. The only exception is the first T in each oligomer, which is cut at higher frequency than surrounding sequences, perhaps indicating an unusual structural environment for this nucleotide.

Methods. Purified plasmids²¹ were linearized with *Hind*III and *Eco*RI and 3'-labelled²⁰ with ³²P at the *Hind*III end. After reaction with hydroxyl radical⁸, the reaction products were denatured and electrophoresed on an 8% polyacrylamide/8 M urea gel²⁰. Bands were assigned by reference to products of a Maxam-Gilbert G + A sequencing reaction²² run on an adjacent lane of the gel. Hydroxyl-radical cleavage leaves 5' phosphate ends, the same as Maxam-Gilbert chemistry²². The assigned base corresponds to the deoxyribose that was attacked by the hydroxyl radical.



ated with the narrow minor groove in the AATT sequence are the large positive propeller twists of the base pairs¹² and an ordered water structure ('spine of hydration')¹³ in the minor groove. Changes in sequence that affect these structural features lead to widening of the minor groove¹². For example, guanine, with its 2-amino group in the minor groove, interferes with the spine of hydration^{12,13}. Interruption of an adenine tract by a guanine (AAGAA) abolishes bending⁴.

In the case of the two adenine tracts considered in this paper, the sequence differences are more subtle. One obvious difference is the presence of a 5'-TpA-3' step in the sequence with normal gel mobility⁶, instead of a 5'-ApT-3' step in the bent sequence. Recent calculations^{14,15}, supported by the Calladine-Dickerson rules^{16,17} for B-DNA structure, have indicated that the TpA step will have a lower propeller twist because of steric clash in the minor groove between cross-strand adenines. A smaller propeller twist would lead to a wider minor groove¹². Nuclease digestion studies¹⁸ suggest that the TpA step has a wider minor groove than the ApA or ApT step. It was shown that interruption of an adenine tract by a single thymine (the sequence AATAA, with one TpA step) eliminates bending⁴.

Our results call into question key assumptions of the newest version of the wedge model⁷ that was designed to account for the gel mobility differences⁶ of $(GAAAATTTTC)_n$ and $(GTTTTAAAAC)_n$. The original wedge model¹⁹ postulated that the ApA dinucleotide forms a 'wedge' which, when phased with the helix screw, causes DNA to bend. By assuming particular values of roll and tilt angles for an AA dinucleotide wedge, Ulanovsky and Trifonov⁷ found that simple vector addition of the roll and tilt of individual AA dinucleotides sufficed to rationalize the gel mobilities of a variety of adenine-tract sequences. If these vectors happen to cancel (as was proposed⁷ for $(GTTTTAAAAC)_n$), the DNA molecule is predicted to lack global curvature. We emphasize, though, that according to the wedge model^{7,19} the local conformation of an AA dinucleotide is the same for any sequence, curved or straight; it is only the summation of these local structural anomalies that yields curvature.

In contrast, the gradient of hydroxyl-radical cutting frequency along an adenine tract in bent DNA (Fig. 2*a*, and ref. 8) is

evidence that each adenine is in a slightly different structural environment. Also we have shown here that an adenine tract is capable of adopting a conformation different from that associated with bent DNA⁸. Therefore, no cancellation of wedge angles⁷ is necessary to explain the normal gel mobility of the CGTTTTAAAA polymer. Our experiments show that this sequence lacks a particular structural feature characteristic of bent DNA, and for this reason is unlikely to be bent.

The details of the structures that are actually adopted by the two sequence isomers are not as important as the observation that there is a clear dependence of the structure of an adenine tract on its sequence context. We offer one possible explanation for this structural difference, a change in groove shape, which is supported by other structural data.

The roll and tilt angles (8.4° and 2.4°) of the most recent version of the wedge model⁷ were calculated by assuming that the wedge vectors of GTTTTAAAAC cancel. As we have shown here that the adenine tracts in CGTTTTAAAA do not adopt the same structure as other adenine tracts, quantitative application of these wedge angles⁷ to other sequences should be reassessed. Detailed structural studies of a wide variety of DNA sequences will be necessary before we can construct a general theory to describe DNA bending.

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Multiple liquid crystal phases of DNA at high concentrations

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DNA packaging *in vivo* is very tight, with volume concentrations approaching 70% w/v in sperm heads, virus capsids and bacterial nucleoids¹⁻³. The packaging mechanisms adopted may be related to the natural tendency of semi-rigid polymers to form liquid crystalline phases in concentrated solutions⁴⁻⁸. We find that DNA forms at least three distinct liquid crystalline phases at concentrations comparable to those *in vivo*, with phase transitions occurring over relatively narrow ranges of DNA concentration. A weakly birefringent, dynamic, 'precholesteric' mesophase with microscopic textures intermediate between those of a nematic and a true cholesteric phase forms at the lowest concentrations required for phase separation. At slightly higher DNA concentrations, a second mesophase forms which is a strongly birefringent, well-ordered cholesteric phase with a concentration-dependent pitch varying from 2 to 10 μm . At the highest DNA concentrations, a phase forms which is two-dimensionally ordered and resembles smectic phases of thermotropic liquid crystals observed with small molecules.

Although specialized proteins are involved in many DNA packaging processes, descriptions of mechanisms of DNA packaging must also consider the intrinsic tendency of the stiff DNA chain to fold when confined to a small volume. Stiff non-electrolyte polymers form highly ordered, liquid crystalline phases above a critical concentration dependent on the persistence length⁴⁻⁸. Formation of nematic or cholesteric liquid crystalline phases by semi-rigid polymers, such as polybenzyl-L-glutamate, in organic solvents has been studied in detail^{7,8}, and was predicted theoretically by Onsager⁴, Flory^{5,6} and others. Much less is known about the behaviour of semi-rigid polyelectrolytes. Because DNA, fibrous proteins and certain polysaccharides are polyelectrolytes involved in numerous supra-molecular associations *in vivo*, an understanding of the phase behaviour of these biopolymers is of fundamental biological importance.

Ordering of semi-rigid polymers at high concentrations occurs spontaneously to minimize the macromolecular excluded volume⁴⁻⁸. Semi-rigid polyelectrolyte behaviour is complicated by charge-shielding requirements. A strong polyelectrolyte is surrounded by a counterion layer which determines the effective axial ratio and excluded volume⁹⁻¹³. Because polymer phase behaviour depends on the effective polymer dimensions, the critical concentration for DNA ordering is a sensitive function of ionic strength and counterion type.

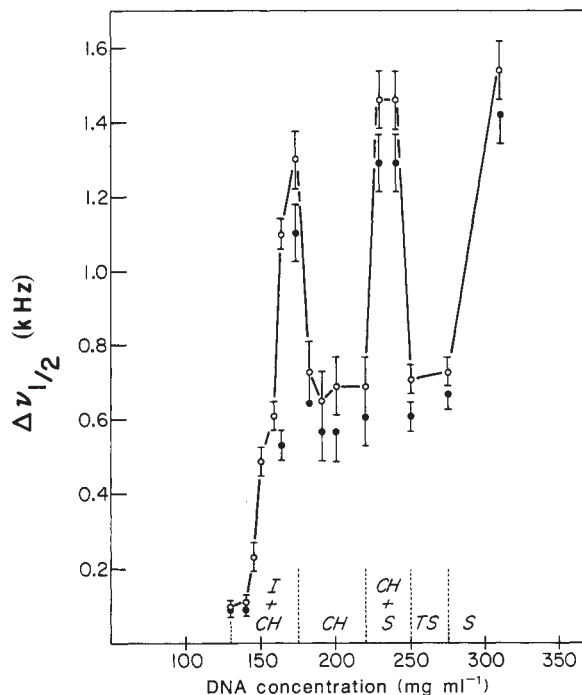


Fig. 1 Dependence of solid state ³¹P NMR linewidth on DNA concentration suggesting multiple phase transitions. DNA fragments of average length 146 base pairs (~500 Å) and with a narrow distribution about this length were prepared by digestion with micrococcal nuclease of calf-thymus chromatin previously depleted of histone H1, and by subsequent deproteinization¹⁶. Short DNA molecules are studied as a preliminary step to understanding the natural behaviour of DNA at *in vivo* concentrations. Such defined samples are useful because effects of relative molecular mass heterogeneity are minimized and phase transitions are sharp and kinetically rapid. Using DNA fragments of this size is appropriate because the driving forces are the same for ordering of high axial ratio, rod-like molecules and long semi-flexible polymers of identical composition^{5,6}. Open circles: data taken at 30°C; filled circles: data taken at 50°C. Spectra were obtained on non-spinning samples at a phosphorus frequency of 61.3 MHz on the 'Seminole', an in-house constructed multinuclear Fourier transform (FT) spectrometer equipped with wide-bore superconducting solenoid and quadrature detection, modified for solid-state applications. Sweep width was ±25 kHz, pulse repetition times were 3 s, and 600 scans were added for each spectrum. Gated proton decoupling was applied during data acquisition.

Previous studies demonstrated that aqueous solutions of persistence length DNA (~500 Å), with 0.3 M NaCl as the supporting electrolyte, form biphasic liquid crystalline solutions containing spherulites at DNA concentrations (C_{DS}) of 130-170 mg DNA ml⁻¹ at room temperature, and become fully liquid crystalline at higher C_D (refs 14-16). Phase transition boundaries were in good agreement with predicted rigid rod behaviour when DNA was treated as a scaled rod with an effective radius of 21.5 Å at this ionic strength¹⁶. Here we report the phase behaviour of DNA solutions over a range of C_{DS} from 100 to 350 mg DNA ml⁻¹, as determined by solid-state ³¹P NMR spectroscopy, polarized light microscopy and by electron microscopy.

Solutions showed a uniform liquid crystalline phase at C_{DS} from 170 to 220 mg ml⁻¹. A C_D of 170 mg ml⁻¹ corresponds to an effective DNA volume fraction of 0.76, assuming 21.5 Å for the effective DNA radius¹⁶. Extrapolation to higher C_{DS} yields an apparent effective volume fraction >1.0 for C_{DS} exceeding ~230 mg ml⁻¹. As DNA solutions were prepared with C_{DS} over