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The B–Z transition in supercoiled DNA depends on sequence beyond nearest-neighbors

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ABSTRACT

In order to examine sequence-dependent structural effects in DNA, the ability of alternating purine-pyrimidine fragments to undergo a B-Z transition when cloned in a supercoiled plasmid was determined solely as a function of sequence, with base and nearest-neighbor composition held constant. Sequences of 22 GC and 2 AT base pairs were synthesized such that the AT base pairs varied between contiguous placement and separation by eight GC base pairs. Results show, surprisingly, that the ease of the B-Z transition varies with the position of the two AT base pairs, occurring at lower superhelical densities when AT base pairs are contiguous, and at higher torsional strain when the AT base pairs are moved further apart.

INTRODUCTION

Our laboratory is interested in sequence-dependent DNA structure. A question in this area is whether transitions between different DNA conformations are truly sequence-dependent, depending not only on the base composition of the DNA, nor even simply on nearest-neighbor interactions, but also on sequence arrangements beyond nearest-neighbors. If such effects could be demonstrated they would constitute evidence for the propagation of structural effects in DNA, either through changes in base stacking or solvent structure, away from the local point of perturbation. The B-Z transition is a particularly well suited system for approaching such questions since the transition is well-defined, easy to follow by a variety of experimental approaches, and inducible at room temperature under mild solvent conditions.

The B-Z transition was first observed by Pohl & Jovin (1) in the alternating polymer poly d(G-C)·poly (dG-C). Since then the Z conformation has also been observed in a number of other synthetic polydeoxynucleotides, in sequences cloned in supercoiled plasmids, and in crystallized oligonucleotides, with many containing AT as well as GC base pairs (reviewed in 2). It has been noted that the difficulty of achieving the B-Z transition increases as the percentage of AT base pairs in the test sequence increases.

Recently we synthesized four different polymers containing 16 base pair (bp), alternating purine-pyrimidine repeating units (3). Each of the polymeric repeating units consisted of fourteen GC base pairs and two AT base pairs. Contrary to what we initially expected, it was shown that the salt concentration required to induce the B-Z transition for the polymer in which the AT base pairs were contiguous was lower than that required when the AT base pairs were separated. The free energy of the salt-induced conformational transition differed by as much as 2.4 kcal/mol, depending on the spacing of the AT base pairs. However, since the behavior of long synthetic polymers in concentrated salt solution may not reflect the behavior of shorter sequences joined to normal B form DNA at physiological

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Table 1. Oligonucleotides synthesized and cloned for this study.

sequence	abbreviation
GATCGCGCGCGCGCGTACGCGCGCGCGC	T(GC)$_0$A
GATCGCGCGCGCGCACGTGCGCGCGCGC	A(CG)$_1$T
GATCGCGCGCGCGTGCGCACGCGCGCGC	T(GC)$_2$A
GATCGCGCGCGCACGCGCGTGCGCGCGC	A(CG)$_3$T
GATCGCGCGCGTGCGCGCGCACGCGCGC	T(GC)$_4$A

ionic strengths we decided to investigate the ability of cloned sequences to undergo the B-Z transition as a function of superhelical density and sequence. The cloned sequences behave similarly to the synthetic polymers in that contiguous AT base pairs are more easily converted to the Z form than separated AT base pairs. These experiments constitute supporting evidence for longer-range, sequence-dependent structural effects in DNA.

MATERIALS AND METHODS

Materials

Nucleotide phosphoramidites and related supplies for chemical synthesis of oligonucleotides were purchased from Applied Biosystems. T4 DNA ligase and T4 polynucleotide kinase

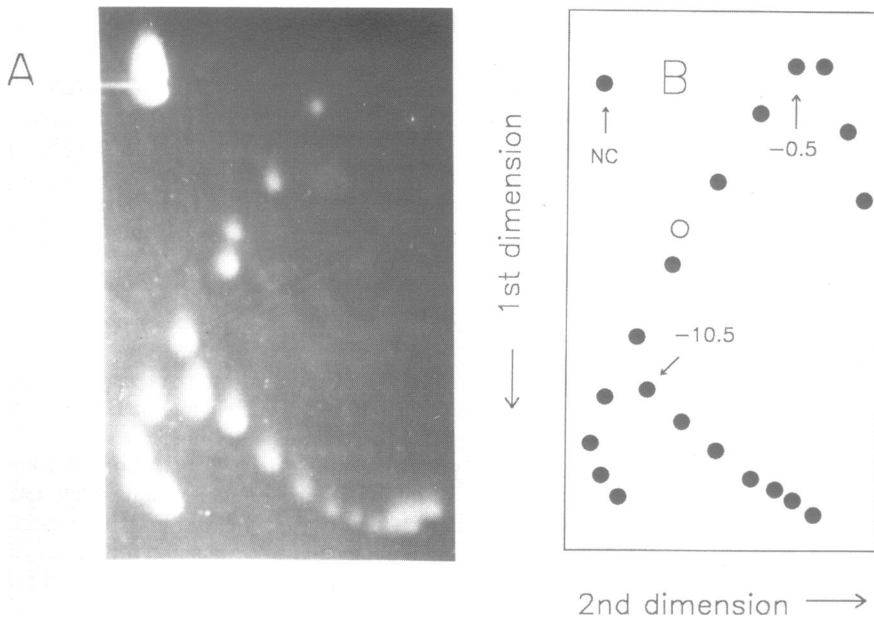

Figure 1. A) Two dimensional gel electrophoresis of topoisomers of pUC18 containing insert T(GC)$_0$A. B) Plot of gel in A. NC is nicked circular plasmid DNA. The open circle marks a small spot found in each gel that runs somewhat off-line, probably reflecting a small conformational transition in the parent plasmid.

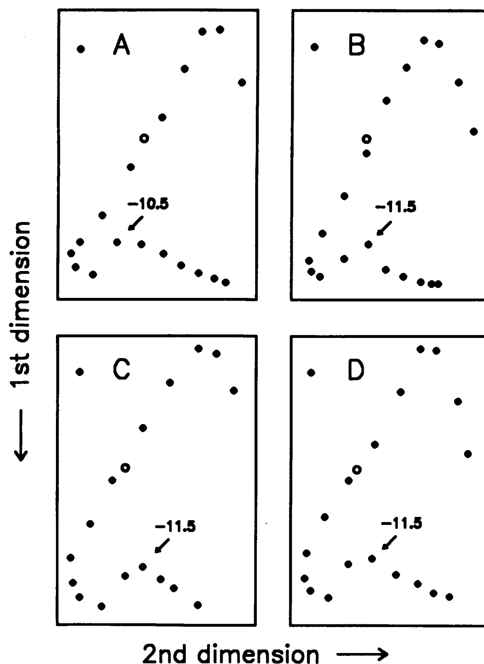

Figure 2. Two dimensional gel electrophoresis of topoisomers of pUC18 containing inserts A) $A(CG)_1T$; B) $T(GC)_2$ A; C) $A(CG)_3T$; D) $T(GC)_4$ A.

were obtained from United States Biochemicals. Restriction enzymes, topoisomerase I, and plasmid pUC 18 were purchased from BRL. *E. Coli* JM 109 cells were purchased from Stratagene and pZ523 plasmid purification columns came from 5′→3′, Inc. Chloroquin and ethidium bromide were from Sigma chemical company and reagents for oligonucleotide sequencing were purchased from Aldrich.

Synthesis of oligonucleotides

Oligonucleotides were synthesized on an Applied Biosystems Model 381A DNA synthesizer using phosphoramidite chemistry. The oligomers were designed to self-anneal in aqueous solutions to yield duplexes with BamH1-compatible sticky ends. Oligomers were purified by preparative gel electrophoresis on 24% polyacrylamide gels containing 7 M urea. Table 1 gives a listing of the oligonucleotides synthesized for this study. Excluding the BamH1 compatible ends the alternating purine-pyrimidine regions are all 24 bp in length.

Construction of recombinant plasmids

The purified oligomers (2 μg) were phosphorylated at 37°C for 2 hours with 30 units of T4 polynucleotide kinase in a buffer of 50 mM Tris·HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM spermidine and 0.1 mM EDTA. Fragments were ligated in the same buffer with 1 unit of T4 DNA ligase at 4°C for 20 hr into BamHI-cleaved, dephosphorylated pUC18. *E. coli* JM109 cells were made competent by incubation with cold 50 mM $CaCl_2$ and 100 μl of the competent cells were transfected with 20 ng of recombinant plasmid using standard methods (4). Successful transformants were detected by growth on agar plates containing ampicillin/X-gal. Transformed colonies were picked, grown up in 1 liter batches, and the plasmid DNA partially purified by alkaline lysis (4).

The plasmid DNA was further purified to electrophoretic homogeneity using pZ523 plasmid purification columns (5′ → 3′ Inc). The identity of the inserts was subsequently confirmed by Maxam-Gilbert sequencing (5).

Two dimensional gel electrophoresis

A population of topoisomers was generated in a manner similar to that of Singleton & Wells (6) for plasmids containing each of the sequences listed in Table 1. In the general case 1 μg of plasmid DNA in each of 10 test tubes, each containing different ethidium bromide concentrations ranging from 0.5−10 μM, was incubated with 2 units of topoisomerase I in 50μ l reaction mixtures containing 50 mM Tris·HCl, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.1 mM EDTA, and 30 μg/ml bovine serum albumin. The topoisomers were then mixed, phenol extracted, precipitated with ethanol, and loaded onto the corner of a 1% agarose gel with dimensions of 24×20×0.4 cm. Electrophoresis in the first dimension was carried out at 100 V for 15 hours using 89 mM Tris, 89 mM boric acid, 2 mM EDTA (TBE) as the running buffer. The gel was then soaked for 8 hr in TBE buffer containing 0.75 μg/ml chloroquine and electrophoresis continued in the second dimension at 80 V for 13 hours in TBE buffer with the same concentration of intercalator. The gel was stained with ethidium bromide and photographed under 300 nm ultraviolet light.

RESULTS

Figure 1A shows the results of two -dimensional (2D) gel electrophoresis of a topoisomer distribution of plasmid pUC18 containing insert $T(GC)_0A$, while Fig. 1B is a drawing of the gel to convey the information more clearly. A discontinuity in mobility occurs between topoisomers −9.5 and −10.5, indicating that a conformational change has occurred in the supercoiled plasmid at that point. The measured decrease in mobility in the first dimension of plasmids with topoisomer numbers of −10.5 or lower corresponds to a change in writhe of 4.2. The expected change in writhe due to a length of DNA undergoing a B-Z transition is

$$\Delta W = l/10.5 + l/12 \qquad [1]$$

where l is the length in base pairs (here 24) of the region undergoing the conformational transition. In this instance, then, ΔW is expected to be 4.3. Thus we conclude that we are observing a B-Z transition. The free energy of the transition can be calculated using the relationship (7−9)

$$\Delta G_{B\text{-}Z} = \frac{1100 \cdot RT \cdot}{N} (\Delta\tau_B^2 - \Delta\tau_Z^2) \qquad [2]$$

where the empirical constant 1100 reflects the torsional rigidity of DNA (7−9), R is the gas constant in cal/mol-deg, T is the absolute temperature, N is the length of the plasmid in base pairs, $\Delta\tau_B$ is the linking difference, $\alpha - \alpha_0$, at the midpoint of the B-Z transition, and $\Delta\tau_Z = (\Delta\tau_B + \Delta W)$ (9). With the midpoint taken to be $\Delta\tau_B = -10$, the calculation yields a free energy change of 16.0 kcal/mol for the B-Z transition. This value is very close to that obtained when the same calculation is carried out on data for a similar, published sequence, 2 GC base pairs shorter (10).

Similar 2D electrophoreses of plasmids containing inserts of the other sequences from

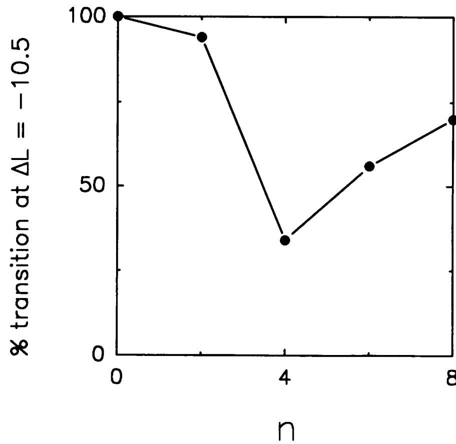

Figure 3. Percentage of B-Z transition occurring by topoisomer −10.5 versus n, the number of GC base pairs separating the two AT base pairs in the cloned fragments.

Table 1 are shown in Fig. 2A−D. In sequence $A(CG)_1T$ the B-Z transition also occurs between topoisomers −9.5 and −10.5. For sequences $T(GC)_2$ A, $A(CG)_3T$, and $T(GC)_4A$, however, the topoisomer number at which the B-Z transition occurs changes, even though these sequences have the same base composition as sequence $T(GC)_0A$ and the same nearest-neighbor composition as $A(CG)_1T$. For these plasmids the transition occurs with a midpoint near topoisomer −10.5. Thus from equation 2 it can be calculated that the value of $\Delta G_{B\text{-}Z}$ for the free energy of the B-Z transition in a cloned 24 bp alternating purine-pyrimidine sequence containing 2 AT base pairs and 22 GC base pairs can vary by 1.1 kcal/mol, or a factor of 6-fold in equilibrium constant, depending simply on the relative positioning of the AT base pairs. To put this number in perspective, the extra cost to the B-Z transition of exchanging one AT base pair for one GC base pair is about 0.9 kcal/mol (11,12). Thus changing the sequence of the cloned insert can have an effect on the energetics of the B-Z transition greater than changing its base composition.

A close examination of Fig. 2 shows that the midpoint of the transition continues to change as the AT base pairs are moved further apart. Fig. 3 illustrates this by plotting the percentage of B-Z transition, as measured by the change in writhe, that has occurred by topoisomer −10.5 vs. n, the number of nucleotides separating the AT base pairs. A minimum occurs when they are four nucleotides apart. Thus it seems that the structural perturbation induced by an AT base pair in a sequence of alternating GC base pairs can extend over 4 nucleotides, or about 13.5 Å.

DISCUSSION

Many of our conceptions about DNA structure have had to be modified in the past decade, mainly as the result of X-ray diffraction studies of single crystals of oligonucleotides. Thus it has been demonstrated that the conformation of DNA is actually quite flexible and the structural complexity of DNA is now generally recognized. One simplification in thinking about the structure of DNA that remains popular, however, is that the physical properties of double stranded polydeoxynucleotides are just the sum of nearest-neighbor interactions.

Much of the impetus for this idea came from early work successfully predicting spectroscopic or melting properties of synthetic polynucleotides (13−15). Those studies, however, were carried out with simple repeating polymers since the chemical synthesis of DNA was, at that time, still an arduous task. The current ease of synthesis of complex sequences allows a re-investigation of the question of sequence-dependent DNA structural transitions.

We chose to examine the B-Z transition since it has been well-studied and is easy to follow experimentally. The two AT base pairs per 24 bp alternating purine-pyrimidine, cloned fragment listed in Table 1 are separated by an increasing distance. In all sequences the base composition is identical, and in all but the first sequence the nearest-neighbor compositions are also identical. The first sequence differs from the remaining ones only in having one TA and one CG nearest-neighbor pairs substituted for one CA and one TG. The difference in stacking energy associated with this change is expected to be only ~0.3 kcal/mol (16), with the former neighbor pairs more stable in the B form, and the expected difference in melting temperatures is ~0.07 °C (17). Nonetheless Figures 1 & 2 clearly show that the degree of supercoiling required to induce the B-Z transition can differ by as much as 1.1 kcal/mol. Thus the results reported here show that conformational transitions in complex sequences, unlike those in simple repeating polymers, can not alway be accounted for as sums of nearest-neighbor interactions.

What are the differences in structure between the oligomers that give rise to their differences in ability to undergo the B-Z transition? This is a difficult question to approach experimentally but several general inferences can be made. The differences appear to be on the B side of the transition since changes in melting behaviour (B-coil transition) of oligonucleotides with sequences similar to the cloned fragments examined here have also been observed (Luthman & Behe, unpublished). Since stacking interactions seem unable to account for the differences (16,17), and since backbone conformations are also unlikely to have a large effect (18), the major remaining factor is solvation. It may be that the greater ability of AT base pairs to bind solvent (19) can partially order water molecules several base pairs removed, and that separating two AT base pairs by several nucleotides increases the total number of bound solvent molecules over that found with two contiguous AT base pairs.

The results reported here may have implications beyond the B-Z transition. For example, cellular processes which require a change in DNA conformation, such as the initial recognition events in transcription and replication, may depend sensitively on sequence in the manner described above. It would seem, now that the synthesis of complex DNA sequences is possible, that further investigation into the sequence dependence of DNA conformational transitions is warranted.

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