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The non-B-DNA structure of d(CA/TG)$_n$ differs from that of Z-DNA

(left-handed DNA/DNA supercoiling/torsional stress/rat prolactin/repetitive DNA)

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ABSTRACT **Chemical probing of two predominantly alternating purine-pyrimidine d(CA/TG)$_n$ repeats led us to propose previously that in supercoiled plasmids these elements adopt a non-B-DNA structure distinct from that of Z-DNA formed by d(CG)$_n$ sequences. Here, we present further evidence supporting this contention. Reactivity with the conformation-sensitive reagent chloroacetaldehyde, which reacts with unpaired adenines and cytosines, was confined strictly to adenines in the d(CA/TG)$_n$ repeat. In contrast, only bases outside the d(CG)$_n$ repeat exhibited chloroacetaldehyde reactivity. Two-dimensional gel analysis of topoisomers containing d(CA/TG)$_n$ tracts with bases out of strict purine-pyrimidine alternation revealed multiple superhelical-dependent transitions to an alternative left-handed structure. Within individual plasmid molecules, these multiple transitions resulted from the stepwise conversion of contiguous segments of alternating purine-pyrimidine sequence, which are delimited by bases out of alternation, to the full-length alternative conformation. When the left-handed helices increased in length to include more bases out of alternation, the average helical pitch changed substantially to produce a less tightly wound left-handed helix. Overall, these data indicate that d(CA/TG)$_n$ tracts adopt a left-handed conformation significantly different from that of the canonical Z-DNA structure of d(CG)$_n$ sequences.**

The repetitive sequence (dC-dA)$_n$·(dT-dG)$_n$ [referred to as d(CA/TG)$_n$] is relatively common within the eukaryotic genome, occurring about once every 50–100 kb of DNA or approximately once per chromosomal domain (1). There is evidence that d(CA/TG)$_n$ repeats induce (2) or repress (3, 4) transcription, stimulate recombination (5–7), and contribute to genomic instability (8), which may be linked to colorectal cancer in humans (9, 10). The functional consequences of d(CA/TG)$_n$ tracts may result from a left-handed non-B-DNA structure that is stabilized by sufficient negative supercoiling (11–13). It has become widely accepted that this left-handed conformation is equivalent to the x-ray crystal structure of Z-DNA obtained from a d(CG)$_n$ oligomer (14).

Several observations have led us to reexamine the non-B-DNA structure adopted by d(CA/TG)$_n$ repeats. The conformation-sensitive reagents S1 nuclease (4, 15, 16), osmium tetroxide (17, 18), potassium permanganate (19), hydroxylamine (20), chloroacetaldehyde (CAA) (21), and bromoacetaldehyde (16, 21) react throughout the non-B-DNA structure of d(CA/TG)$_n$ repeats, whereas these reactivities are confined at B–Z junctions for d(CG)$_n$ repeats (11, 20, 22). It was proposed (16, 23) that the d(CA/TG)$_n$ sequence adopted a left-handed conformation dissimilar to the canonical d(CG)$_n$ Z-DNA sequence. In addition, we previously observed nonuniform reactivity of diethyl pyrocarbonate throughout the non-B-DNA structure of d(CA/TG)$_n$ tracts that suggested pronounced helical variation along the non-B-DNA structure (19). Thus, by definition, the overall structure could not be that of a perfect Z-type helix.

Several physical studies also support the position that d(CA/TG)$_n$ sequences adopt a left-handed structure distinct from the prototypical d(CG)$_n$ Z-DNA conformation. In fact, alternative left-handed helices have been proposed (24–27). In general, the conditions of the right-to-left transition are more stringent for d(CA/TG)$_n$ sequences and its derivatives than they are for d(CG)$_n$ sequences. For example, unlike the d(CG)$_n$ polymer, which undergoes a B-to-Z transition in concentrated sodium chloride (28, 29), d(CA/TG)$_n$ polymers require methylation at the C-5 position of cytosine and heating to 62.5°C as well as high salt to effect the midpoint of a right- to left-handed transition (30). There is no measurable temperature dependence for the C-5-methylated d(CG)$_n$ polymer (29). Another modification of the cytosine C-5 position, bromination, produces several structural differences between d(CG)$_n$ and d(CA/TG)$_n$ oligonucleotides.

Analyses of d(CG)$_n$ and d(CA/TG)$_n$ sequences in negatively supercoiled plasmids have demonstrated several major differences in the thermodynamics of the right- to left-handed transitions. While the d(CG)$_n$ polymer exhibits a clear all-or-none cooperative transition to the Z conformation (31), only an "initial" B-DNA to non-B-DNA conversion of the d(CA/TG)$_n$ sequence is cooperative (13). The free energy requirements of B/non-B junction formation and the effects of magnesium ions on these junctions differ between the two alternating purine-pyrimidine (APP) sequences (32, 33).

There is also biological evidence for the dissimilarity of the d(CG)$_n$ and d(CA/TG)$_n$ sequences. In an *in vitro* transcription assay, *Escherichia coli* RNA polymerase is able to transcribe through a d(CG)$_n$ sequence when it is in the B form in a supercoiled plasmid. However, at levels of negative superhelicity sufficient to stabilize the Z-DNA conformation, the traversing polymerase was quantitatively blocked at the boundary of the d(CG)$_n$ sequence proximal to the promoter. In contrast, even at a high negative superhelical density, the polymerase was able to transcribe effectively through the left-handed conformation of the d(CA/TG)$_n$ sequence (34). Apparently, this enzyme can distinguish structural differences between the non-B-DNA structures of the two polymers.

In the context of these observations, we have reexamined the nature of the non-B-DNA structure adopted by d(CA/TG)$_n$ repeats under torsional stress. The conformation-sensitive reagent, CAA, reveals major differences in the patterns of reactivity in d(CA/TG)$_n$ versus d(CG)$_n$ sequences. We have also analyzed two d(CA/TG)$_n$ repeats of 58 and 178 bp from the rat prolactin gene by two-dimensional topoisomer gel electrophoresis. Within an individual topo-

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Abbreviations: APP, alternating purine-pyrimidine; CAA, chloroacetaldehyde; HZ, hydrazine/piperidine; FA, formic acid/piperidine.

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isomer series, the conversion from the right- to left-handed structure exhibited multiple DNA transitions and each transition proceeded less cooperatively than has been observed for $d(CG)_n$ sequences that form Z-DNA. Quantitative analysis of the number of superhelical turns relaxed by the left-handed conformation of each $d(CA/TG)_n$ repeat indicated an average left-handed helical pitch that diverged significantly from that of Z-DNA. Thus, these data strongly suggest that $d(CA/TG)_n$ repeats adopt a left-handed structure significantly different from that of the canonical Z helix of $d(CG)_n$ sequences.

MATERIALS AND METHODS

CAA Modification. Plasmid pCG_{16} contains a $d(CG)_{16}$ sequence at the *Bam*HI site of pUC18. Plasmid pCA_{31} containing an insert of $d(CA/TG)_{31}$ at the *Sma* I site of pUC18 was kindly provided by Marc Leng (Centre de Biophysique Moléculaire, Orléans, France). Where indicated, supercoiled DNA was isolated from *E. coli* and modified with CAA in Tris maleate (pH 5 or 7) as described (35). An *Eco*RI/*Bgl* I fragment (uniquely labeled with [γ-^{32}P]ATP at the *Eco*RI site) of pCA_{31} and a *Hin*dIII/*Pvu* I fragment (uniquely labeled with [γ-^{32}P]ATP at the *Hin*dIII site) of pCG_{16} were subsequently reacted with either hydrazine/piperidine (HZ) or formic acid/piperidine (FA) to cleave cytosines and purines, respectively, followed by separation on a urea-denaturing 8% polyacrylamide gel (35).

Two-Dimensional Gel Electrophoresis. Plasmids pBSKS.178 (3272 bp) and pBSKS.58 (3138 bp) are derivatives of pBluescript KS(+) (Stratagene) (19). In all two-dimensional gel electrophoresis experiments, the large repetitive inserts within the plasmids were sequenced (19) to confirm the absence of deletions or rearrangements. Plasmid topoisomers were generated and purified as reported (19). For first-dimension electrophoresis, pooled plasmid topoisomers were loaded into a circular well in 1.5% agarose gels (39 × 40 × 0.5 cm) buffered by either TBE (89 mM Tris borate, pH 8.3/1 mM EDTA) (see Fig. 2) or HTE (25 mM Hepes/1 mM EDTA titrated to pH 7.5 with triethylamine) (see Fig. 3) and electrophoresed at 90 V for TBE gels or at 70–75 V for HTE gels for ≈70 h with constant buffer recirculation (100 ml/min). Temperature (25°C) and pH remained constant at both the anode and cathode throughout electrophoresis. For second-dimension electrophoresis, gels were equilibrated in their respective buffer supplemented with the indicated concentrations of chloroquine diphosphate for 8 h before electrophoresis under conditions identical to the first dimension. After electrophoresis, the gels were soaked for several hours to remove the chloroquine, stained with ethidium bromide, destained in H_2O, and photographed on a short-wave UV light transilluminator.

RESULTS AND DISCUSSION

CAA Detects Differences in $d(CG)_n$ and $d(CA/TG)_n$ Sequences. CAA reacts specifically with unpaired bases at the exocyclic amino group and N-3 of cytosine or N-1 of adenine (36). In Fig. 1 we reacted pCA_{31} and pCG_{16} containing inserts of $d(CA/TG)_{31}$ or $d(CG)_{16}$, respectively, with CAA to probe for structural differences between each insert. CAA-modified dA and dC residues are detected as new cleavage sites in the cytosine (HZ) and purine (FA) cleavage ladders, respectively. In plasmid pCA_{31}, CAA reactivity was strictly confined to adenines within the $d(CA/TG)_{31}$ tract (Fig. 1*A*, HZ, lanes 2–7, arrowheads), whereas cytosines (Fig. 1*A*, FA, lanes 9–11) within the repeat were unreactive as previously observed (21). The presence of magnesium (Fig. 1*A*, lanes 3, 6, and 10) or zinc ions (Fig. 1*A*, lanes 4, 7, and 11) significantly enhanced CAA reactivity at adenines within the $d(CA/TG)_n$ insert. Cytosines in the insert remained nonreactive

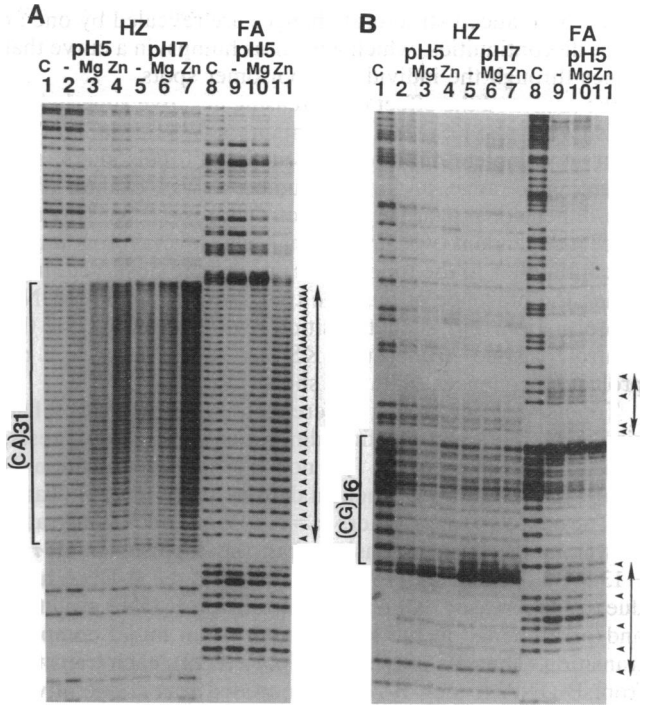

FIG. 1. CAA reactivity of repetitive $d(CA/TG)_n$ (*A*) and $d(CG)_n$ (*B*) sequences. Supercoiled plasmid DNAs ($\sigma = -0.05$) were modified in the absence [lanes 1 and 8 (C, control)] and presence (lanes 2–7 and 9–11) of CAA at pH 5 (lanes 2–4 and 9–11) or pH 7 (lanes 5–7). Sample buffer contained 2 mM Mg^{2+} (Mg), 2 mM Zn^{2+} (Zn), or no metal ion (−), as indicated. Repeats are bracketed on the left. The CAA-modified dA residues in pCA_{31} were detected as new bands in the cytosine cleavage ladder (HZ) in lanes 2–7, as indicated by arrowheads and a double-headed arrow on the right in *A*. CAA reactivity is confined to sequences flanking the $d(CG)_n$ tract as indicated by arrowheads and double-headed arrows on the right in *B*.

with CAA. No additional reactive adenines or cytosines were detected in flanking sequences. Other chemical probes (potassium permanganate, diethyl pyrocarbonate, and dimethyl sulfate) failed to react with sequences that flank similar $d(CA/TG)_n$ repeats in plasmids of very high superhelicity (19). In contrast, plasmid pCG_{16} showed CAA reactivity with adenines (Fig. 1*B*, HZ, lanes 2–7) and cytosines (Fig. 1*B*, FA, lanes 9–11) located in flanking sequences outside the $d(CG)_n$ repeat. This is the typical unpairing pattern previously observed at the B–Z junction (22). The CAA reactivity of these residues was unaffected by the presence of Mg^{2+} ions (Fig. 1*B*, lanes 3, 6, and 10) or Zn^{2+} ions (Fig. 1*B*, lanes 4, 7, and 11).

$d(CA/TG)_n$ Tracts Containing Bases Out of Alternation Undergo Multiple Transitions to a Non-B-DNA Conformation. Two-dimensional gel electrophoresis (31) enables one to determine the nature of a supercoil-driven transition to a non-B-DNA structure within individual topoisomers. We use the term transition to refer to a region, *n* bp long, that converts from the B form to a non-B-DNA form. Such a region consists of one or more segments that are demarcated by out-of-alternation bases (see Fig. 4) (19). At sufficient levels of negative superhelicity, conversion of B-DNA to a non-B-DNA conformation will remove a characteristic number of negative superhelical turns from individual topoisomers of a plasmid and retard their mobility during the first dimension of electrophoresis. Before electrophoresis in the second dimension, intercalation of chloroquine reduces the level of negative supercoiling below that required to maintain non-B-DNA structures, allowing topoisomers to migrate according to their respective linking numbers. Negative

supercoil-induced structural changes are revealed by one or more discontinuities, which appear as humps, in a curve that traces through the individual topoisomer spots.

We analyzed the effect of increasing negative superhelical density on pBSKS.178 that contains a 178-bp d(CA/TG)$_n$ tract. A complete mixture of pBSKS.178 topoisomers, representing a range of linking numbers, was examined by two-dimensional agarose gel electrophoresis (Fig. 2). From three independent two-dimensional gels, several pronounced discontinuities in the topoisomer curve were observed, indicating the presence of multiple transitions to a non-B-DNA conformation. No such transitions were observed for the control plasmid pBluescript KS(+), which lacks any rat prolactin sequences (data not shown).

Topoisomers at linking differences −12.8 and −13.8 had similar mobilities in the first dimension of electrophoresis, indicating the onset of the first non-B structural transition (I) in pBSKS.178 at a superhelical density, σ, of −0.044 (Table 1). The next two topoisomers (τ = −14.8 and −15.8) migrated slower in the first dimension than the topoisomer at τ = −13.8, suggesting additional unwinding of the B-DNA helix due to an increased extent of a uniform non-B-DNA structure and/or a change in the helical pitch of an initial complete transition. Thus, as previously reported (13), each transition from B-DNA to non-B-DNA proceeded less cooperatively than has been observed for classical Z-DNA sequences of the d(CG)$_n$ variety (31). The regular increase in the mobilities of topoisomers −15.8 to −18.8 indicates that transition I was complete. Tracing through the curve of pBSKS.178 topoisomers further, we see that a second transition (II) began at approximately τ = −18.8 (σ = −0.060; however, due to the relaxation of 6.0 supercoils by transition I, the level of available superhelical density or effective superhelical density was actually −0.041) and appeared complete at about τ = −21.8 to −23.8. A third transition to the non-B-DNA conformation started at τ = −25.8 (effective σ = −0.044). We stress that the relative mobilities of each pBSKS.178 topoisomer are highly reproducible.

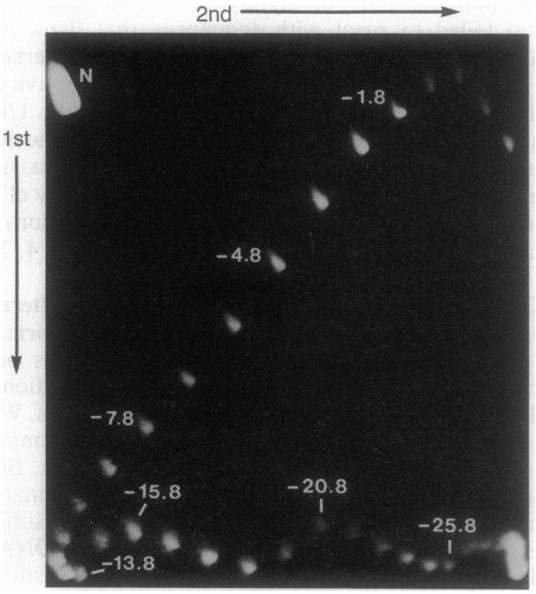

FIG. 2. Two-dimensional electrophoresis of pBSKS.178 DNA topoisomers containing the 178-bp d(CA/TG)$_n$ tract. A mixture of topoisomers of different mean superhelical density were electrophoresed in TBE buffer as described. Chloroquine was included at 1.75 μg/ml during second-dimension electrophoresis. The nicked-circular DNA (N) and linking differences for various topoisomers are indicated.

Table 1. d(CA/TG)$_n$ tract transitions and helical repeats

CA/TG tract	Trans.	n	$-(\alpha - \alpha_0)$	$-\sigma$	−ΔTw B–Z	−ΔTw Obs	h*
178 bp†	I	35	13.8	0.044	6.3	6.0	13.1
	II	74	18.8	0.060	13.2	12.0	14.9
	III	122	≈25.8	0.083	21.8	≈17.8	19.7
178 bp‡	I	35	13.5	0.044	6.2	5.8	13.8
	II	74	20.0	0.065	13.1	11.0	18.1
	III	122	≈25.0	0.082	21.6	≈17.0	21.8
	IV	147	≈32.5	0.107	26.0	≈20.5	21.7
	V	178	≈35.0	0.115	31.5	≈25.0	21.3
58 bp†	I	34	13.2	0.044	6.1	6.0	12.3
	II	58	18.2	0.060	10.4	8.9	17.2

Experimental quantities as derived from two-dimensional topoisomer gel electrophoresis. Number of base pairs (n) that have undergone each transition (Trans.; roman numerals) as determined from chemical probing (19) is indicated. ($\alpha - \alpha_0$) and σ are the cumulative critical specific linking differences and cumulative superhelical densities, respectively, at which each transition initiated; however, all transitions initiated when the level of available superhelical density was −0.045 ± 0.003 (mean ± SD; see *Results and Discussion*). ΔTw refers to changes in twist experimentally observed (Obs; as measured by relaxation of supercoils) or predicted when a region of length n converts to canonical Z-DNA (B–Z). Average helical repeat, h, is indicated for each d(CA/TG)$_n$ repeat at the completion of each individual transition.

*Values can be recalculated relative to the winding number (37) using helical repeats for B-DNA of 11.0 and 11.2 bp per turn in TBE and HTE buffers, respectively.

†Electrophoresis in TBE buffer. Helical repeats of B- and Z-DNA of 10.5 and 12.0 bp per turn, respectively, were used to calculate $-\Delta Tw_{B-Z}$.

‡Electrophoresis in HTE buffer. Helical repeat of B-DNA was estimated at 10.7 bp per turn by the band counting method of Keller (38) and that for Z-DNA was assumed to be unchanged.

To demonstrate that this multiple transitional behavior was not an anomalous property of the 178-bp d(CA/TG)$_n$ tract, we also performed two-dimensional gel electrophoresis on pBSKS.58, which contains a 58-bp d(CA/TG)$_n$ repeat. From five independent two-dimensional gels, we observed two distinct and sequential transitions (designated I–II; see Fig. 4) to a non-B-DNA structure (Table 1). Thus, the less cooperative nature of individual transitions to non-B-DNA is a general property of d(CA/TG)$_n$ repeats.

To validate comparisons of two-dimensional gel data obtained in TBE buffer with chemical probing data performed in HTE buffer (19), we performed two-dimensional analyses of pBSKS.178 topoisomers in a gel buffered with HTE (Fig. 3). More chloroquine was included in the HTE buffer during second-dimension electrophoresis in order to resolve topoisomers with large negative linking differences. A simplified tracing of the five observed transitions to non-B-DNA structure as deduced from the topoisomer migration pattern is shown (Fig. 3 *Inset*). To reasonably determine the negative linking difference of the individual topoisomers where each transition to non-B-DNA initiated, several additional experiments were performed in which the chloroquine concentration was varied to resolve different regions of the topoisomer curve (data not shown). The critical superhelical densities ($-\sigma$; Table 1) at the initiation of transitions I–III are in excellent accord for the two buffers, allowing a comparison of data obtained from either buffer system.

For both d(CA/TG)$_n$ tracts in either buffer system, after initiation of the first transition to the non-B-DNA conformation, topoisomers at successively higher superhelical densities underwent additional transitions with concomitant loss of supercoils. Using chemical probes, we observed (19) that chemical reactivities for initial transitions were strictly localized to one end of a d(CA/TG)$_n$ tract and spread contig-

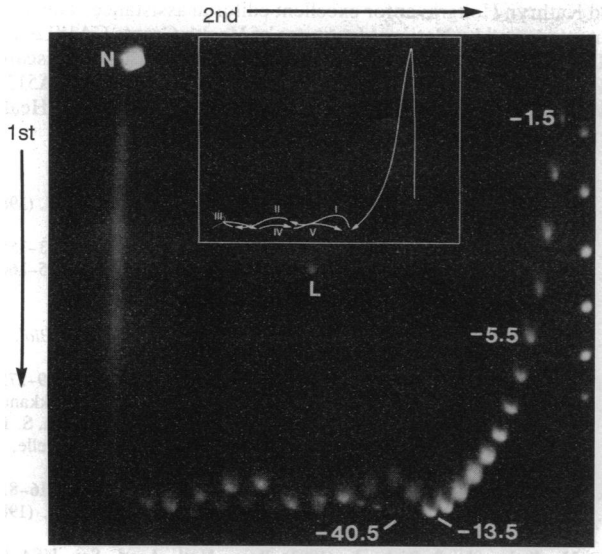

FIG. 3. Two-dimensional HTE gel electrophoresis of pBSKS.178. The same mixture of DNA topoisomers used in Fig. 2 was electrophoresed in HTE buffer as described. The chloroquine concentration during second-dimension electrophoresis was 10 μg/ml. The nicked-circular DNA (N), linear (L) species, and linking differences for various topoisomers are indicated. (*Inset*) Diagrammatic tracing of the five (I–V) observed DNA transitions.

uously until the left-handed helix predominated over the entire length of each repetitive sequence. This pattern of chemical reactivity suggested that transitions beyond the initial one occur sequentially and contiguously within individual plasmid molecules; otherwise, reactivity should have been observed over the entire repetitive sequence. The topoisomer curves corroborate this assertion; if additional transitions corresponded to non-B-DNA conversion of noncontiguous spans of d(CA/TG)$_n$ sequence, then multiple conformers would be visible at the position of each topoisomer containing non-B-DNA structure (39). Thus, the number of base pairs converted to its non-B-DNA form in any one transition corresponded to one or more adjacent, perfectly alternating d(CA/TG)$_n$ segments delimited by bases out of strict purine-pyrimidine alternation (19) (Fig. 4).

The Average Helical Repeat of d(CA/TG)$_n$ Tracts Differs Significantly from That of Z-DNA. By relating the results obtained by two-dimensional agarose gel electrophoresis to chemical probing data (19), we can determine the extent (n bp) and corresponding segments converted to the non-B-DNA structure when each transition is completed (Table 1; Fig. 4). Chemical probing of a "native" population ($\tau = -9.2$ to -22.2) of pBSKS.178 topoisomers demonstrated that plasmids having the highest negative superhelicity stabilized 74 bp of the 178-bp d(CA/TG)$_n$ tract in the non-B-DNA structure. Using a value of 10.7 bp per helical turn for B-DNA in HTE buffer as determined by the band counting method of Keller (38) (data not shown), $\tau = -22.2$ corresponds to a superhelical density of -0.076, which is sufficient to stabilize transitions I and II or 74 bp in the altered conformation. Similarly, for pBSKS.58, chemical probing of a native topoisomer fraction verified stabilization of the entire 58-bp d(CA/TG)$_n$ tract in the non-B-DNA conformation.

We quantitatively determined the number of supercoils relaxed by each completed transition to non-B-DNA (Table 1, $-\Delta Tw_{obs}$). These values can be used to obtain a reasonable estimate of the helical repeat, h, and the sense of the non-B-DNA structure from the relationship $(n/10.5) + (n/h) = \Delta Tw_{obs}$, where n is the extent in base pairs of a completed transition as determined by chemical probing and 10.5 is the helical repeat of B-DNA in TBE buffer (as indicated above, a value of 10.7 is used for gels electrophoresed in HTE buffer). Therefore, the number of relaxed supercoils is equal to the number of turns of unwound right-handed helix plus the number of turns, if any, of left-handed helix that are formed. Each transition relaxes more supercoils than expected for adopting an unwound helical state and, therefore, indicates the formation of a left-handed helical conformation. However, for both d(CA/TG)$_n$-containing plasmids, electrophoresed in either buffer, there is a deficit in the ΔTw_{obs} relative to that predicted for the B-to-Z transition (ΔTw_{B-Z}) characteristic of a d(CG)$_n$ sequence. Hence, the average helical pitch of the d(CA/TG)$_n$ sequences appears to diverge significantly from 12.0 bp per turn (Table 1). This effect becomes more exaggerated as successive transitions to the left-handed conformation are stabilized. This interpretation remains unchanged if we recalculate the data relative to the winding number, ϕ, as described by Cozzarelli *et al.* (37). We emphasize that our values of ΔTw_{obs} may underestimate the left-handed helical pitch since non-B/B-DNA junctions usually lie outside the non-B-DNA conformation (19, 20, 31).

The increasing helical pitch may be attributable to the incorporation of more bases out of alternation within the lengthening left-handed structure. These bases may create quasi-B-DNA/non-B-DNA junctions that interfere with a tightly wound left-handed helix. Assuming that each out-of-alternation base (see Fig. 4) within a repetitive sequence disrupts the left-handed helix to the same extent, we can estimate the potential amount of junctional perturbation. In TBE buffer, at the completion of transition III for the 178-bp d(CA/TG)$_n$ tract, we observed the relaxation of 4.0 fewer supercoils than expected for a B–Z transition. This relaxation

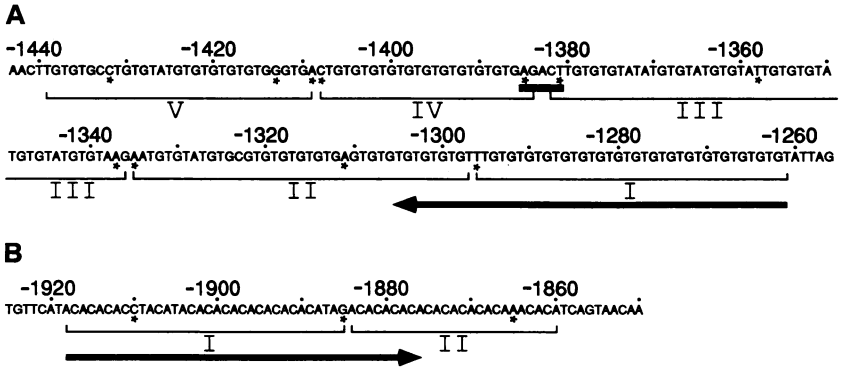

FIG. 4. Summary of transitions to left-handed DNA within d(CA/TG)$_n$ tracts. The 178- (*A*) and 58- (*B*) bp sequences. Brackets labeled with roman numerals indicate distinct transitions from B- to left-handed DNA that encompass one or more segments (19) of perfect APP sequence that are delimited by out-of-alternation bases (asterisks). Arrows indicate directional propagation of successive transitions. A potential junction between two out-of-phase non-B-DNA helices is indicated by the solid bar.

deficit calculates to a potential disruption to left-handed winding of ≈0.8 (4.0/5 bases out of alternation) turn per junction. For the 58-bp tract, we estimate a left-handed helical unwinding of 0.5 turn per junction. The larger value obtained for the 178-bp tract could be due to the different stacking of A·T bp in left-handed DNA helices (40) since all five potential junctions were caused by out-of-alternation A·T bp as opposed to one of three in the 58-bp tract. Despite these differences, the calculated unwinding of the left-handed helix appears to be greater than the right-handed helical unwinding of −0.4 turn per junction reported for B–Z junctions (31). Furthermore, chemical probing demonstrates that perturbations at out-of-alternation bases are well-defined and highly localized, usually occupying only 1–3 bp of DNA (19), making it unlikely that the deficient relaxation of supercoils is completely due to bases out of alternation.

CONCLUSION

We have demonstrated that repetitive $d(CA/TG)_n$ sequences adopt a left-handed helical conformation significantly different from that of Z-DNA assumed by $d(CG)_n$ sequences. The conformation-sensitive reagent CAA revealed major reactivity differences between perfect APP versions of $d(CA/TG)_n$ and $d(CG)_n$ motifs within each repetitive element and in flanking DNA sequences. Two-dimensional gel analysis of topoisomers containing $d(CA/TG)_n$ tracts with bases out of alternation demonstrated several discrete and sequential transitions of contiguous segments (Fig. 4) that produce a left-handed conformation within individual topoisomers. This less cooperative nature of each transition in our topoisomer curves is substantially different than those reported for $d(CG)_n$ sequences. In contrast, transitions to Z-DNA are highly cooperative for $d(CG)_n$ tracts containing bases out of alternation (41, 42) as well as Z–Z junctions (43) between out-of-phase Z helices.

For $d(CA/TG)_n$ tracts, the overall helical repeat increased, and deviated significantly from that of canonical Z-DNA, as each tract propagated to a longer extent to include more bases that disrupted the APP motif. Thus, bases out of purine-pyrimidine alternation appear to disrupt the left-handed helical winding of $d(CA/TG)_n$ sequences. This interpretation is supported by the reactivity of such bases with conformation-sensitive probes (4, 19), suggesting that they occupy positions of significant unstacking and/or phosphate backbone perturbation. Such disruptions in base stacking interactions would be expected to cause a local perturbation in the left-handed helical winding of $d(CA/TG)_n$ sequences; that is, by definition unstacked bases cannot be wound in either a right- or left-handed helix and thus yield an alternative left-handed structure that is not a Z helix.

Our unwinding data cannot distinguish between a transition (*i*) to an unknown non-B-DNA structure that has a uniform state of helical winding intermediate between B-DNA and Z-DNA, and/or (*ii*) a left-handed DNA structure with interspersed helical perturbations. For reasons stated above, we believe that both situations contribute to our observations about $d(CA/TG)_n$ tracts that contain bases out of alternation. At the very least, such sequences do not form contiguous stretches of Z-DNA. The detection of nonuniform reactivity indicative of helical variation along the non-B-DNA structure by potassium permanganate and diethyl pyrocarbonate (19) supports this view. In closing, any interpretation of our data that incorporates either an altered left-handed helical repeat and/or unwinding of a left-handed helix at bases out of alternation underscores our original premise: $d(CA/TG)_n$ repeats form a left-handed non-B-DNA conformation that is significantly different from that of Z-DNA.

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