Cooperativity in A-Tract Structure and Bending Properties of Composite T_nA_n Blocks†

Tali E. Haran and Donald M. Crothers*

Department of Chemistry, Yale University, New Haven, Connecticut 06511

Received December 21, 1988; Revised Manuscript Received February 3, 1989

ABSTRACT: The existence of intrinsically curved DNA molecules incorporating short runs of adenines is undisputed, but none of the current models can explain the entire experimental data set. Recently, Burkhoff and Tullius [Burkhoff, A. M., & Tullius, T. D. (1988) *Nature 331*, 455–457] offered an explanation for Hagerman's observations on $A_4T_4N_2$ vs $T_4A_4N_2$ polymers [Hagerman, P. J. (1986) *Nature 321*, 449–450], which showed that $A_4T_4N_2$ multimers migrate anomalously slowly on polyacrylamide gels and $T_4A_4N_2$ multimers migrate normally. In $A_4T_4N_2$ multimers Burkhoff and Tullius observe a hydroxy-radical cutting pattern associated with bent DNA and a B-like cutting pattern in $T_4A_4N_2$. They attribute this difference in cutting pattern to a clash in the TA step of $T_4A_4N_2$ and suggest that TA_4N_5 might already adopt an unbent B-DNA conformation [Tullius T. D., & Burkhoff, A. M. (1988) in *Structure and Expression. Vol. 3: DNA Bending and Curvature* (Olson, W. K., Sarma, M. H., Sarma, R. H., & Sundaralingam, M., Eds.) pp 77–85, Adenine Press, Guilderland, NY]. We show that the conformation adopted by T_nA_n blocks is similar to that of A_nT_n blocks. Two A-tract structures of opposite polarity coexist in both blocks. Moreover, we demonstrate a cooperative buildup of a T-tract structure adjacent to an A-tract structure that cannot be predicted by any of the current models. We conclude that AA steps do not assume the same conformation in long tracts of A's as in isolated AA steps. Therefore, the assumption of nearest-neighbor models, that global curvature is an additive phenomenon of local effects, is invalid.

The existence of sequence-dependent DNA curvature in fragments containing short runs of adenines (A tracts) has been verified beyond doubt by a variety of techniques (Wu & Crothers, 1984; Diekmann & Wang, 1985; Griffith et al., 1986; Koo et al., 1986; Ulanovsky et al., 1986). Several models have been proposed to explain the phenomenon of DNA curvature on a molecular level. They can be grouped into two main categories: nearest-neighbor models and distal-interaction models. Nearest-neighbor models assume that the conformation of each base-pair step is independent of the conformation of adjacent steps. This group is further subdivided into models that advocate bending based on nearest-neighbor interactions mainly within A tracts ("wedge model"; Trifonov & Sussman, 1980; Ulanovsky et al., 1986; Ulanovsky & Trifonov, 1987) and those proposing bends arising from nearest-neighbor interactions outside A tracts only (Calladine et al., 1988; Srinivasan et al., 1987; Maroun & Olson, 1988).

The wedge model of Trifonov and colleagues (Trifonov & Sussman, 1980; Ulanovsky et al., 1986; Ulanovsky & Trifonov, 1987) assumes that certain dinucleotides, mainly AA, are not coparallel. Each dinucleotide has a characteristic roll and tilt angle associated with it. Repeat of these wedgelike base-pair steps with the helical screw will generate macroscopically curved molecules. The model suggested by Calladine et al. (1988) is a variant of the wedge model, where AA steps are unique in having no roll or tilt and TA steps are unique in having a high roll angle. The other base-pair steps are grouped together with an average roll between these two extremes. Olson and colleagues (Srinivasan et al., 1987; Maroun & Olson, 1988) divide the 16 possible base-pair combinations into base pairs that can roll equally well into the minor and major grooves (symmetric steps) and those with a preference to one groove (asymmetric steps). Only asymmetric steps are responsible for inducing permanent bends into the double helix. The asymmetric steps are all those in which the second position is G or C, as well as the step AT.

Distal-interaction models propose that interactions beyond the nearest-neighbor level may contribute significantly to the local conformation of certain dinucleotides. The main example in this category is the junction model (Marini et al., 1982; Wu & Crothers, 1984; Koo et al., 1986, Koo & Crothers, 1988), which assumes that A tracts, four or more base pairs (bp) long, adopt an alternate non-B-DNA structure. The cause of curvature is the deflection of the global axis of the A-tract structure from that of the adjoining B-DNA region. Another model in this group comes from hydroxy-radical footprinting experiments on DNA. Burkhoff and Tullius (1987) observed that B-DNA is cut by hydroxy radicals with a nearly uniform rate, yet bent kinetoplast DNA is cut with a rate that varies sinusoidally with position. From this they inferred that the minor groove width of the A-tract structure decreases smoothly on going in the 5′ to 3′ direction. Burkhoff and Tullius (1988) suggest that macroscopic curvature of DNA molecules might be linked to this periodic change in the minor groove width of phased A tracts.

Hagerman (1986) made the important observation that multimers of $A_4T_4N_2$ migrate anomalously slowly on polyacrylamide gels. At the same time multimers of $T_4A_4N_2$, which differ from the first sequence by the order of the A and T tracts only, migrate normally. Burkhoff and Tullius (1988) analyzed the cutting pattern of these polymers by hydroxy-radical footprinting and concluded that A_4T_4 fragments adopt a bent conformation, much like that of kinetoplast DNA, while T_4A_4 fragments adopt an unbent, B-DNA-like conformation. According to Burkhoff and Tullius the straight B-like conformation in T_4A_4 is the result of a clash in the minor groove

† This work was supported by National Institutes of Health Grant GM21966 and by Project Grant for Parasitology and Tropical Medicine from the MacArthur Foundation (to D.M.C.).

0006-2960/89/0428-2763$01.50/0

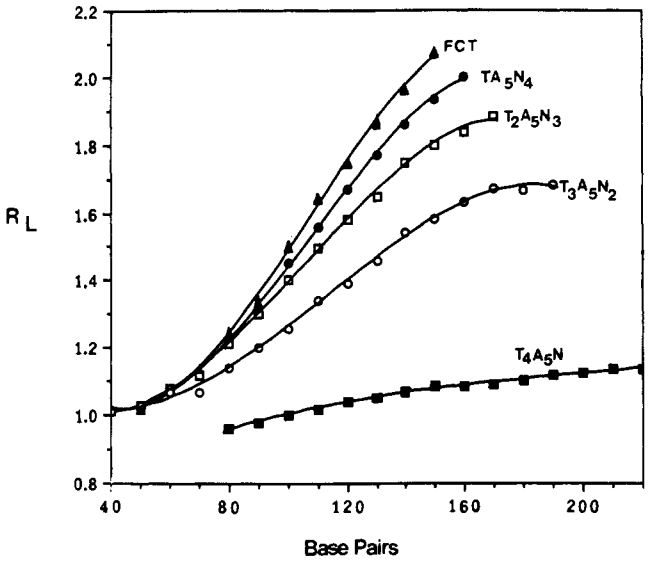

FIGURE 1: R_L values vs actual chain length for series I, $T_nA_5N_{5-n}$ (n = 1–4), and FCT (Table I). R_L is the ratio of apparent chain length (in bp), determined from comparison with size markers on an 8% polyacrylamide gel, to actual chain length (in bp).

at the TA step. This clash prevents the TA step from propeller twisting, a motion known to close down the minor groove of B-DNA (Fratini et al., 1982), and hence at this step the minor groove is forced to remain open.

We have constructed a series of molecules, $T_nA_5N_{5-n}$ (n = 1–4; series I in Table I), and analyzed them by polyacrylamide gel electrophoresis. The questions that we address are the following: (1) What is the structure of T_nA_n blocks? (2) Can a single TA step straighten a curved fragment? (3) If not, when does the change to a straight helix occur? (4) Is this change gradual or is it abrupt? A gradual straightening of the helix in $T_nA_5N_{5-n}$ multimers would support nearest-neighbor models, whereas an abrupt cooperative change to a straight helix would support distal-interaction models. Cooperative transition to A-tract structure was the premise of the original junction model. However, it has never been unambiguously shown before, because the quadratic relation between DNA curvature and its migration on polyacrylamide gels (Koo & Crothers, 1988) hinders the detection of small bends in the double helix. In the present construction this problem is avoided by using the A_5 tract of each fragment as an amplifier to the small bends produced by the adjacent T-tract structure.

Materials and Methods

DNA Synthesis and Purifications. The deoxyoligonucleotides of Table I were synthesized on an automated DNA synthesizer (Applied Biosystems). After synthesis, the oligonucleotides were deprotected and purified, as described before (Haran & Crothers, 1988).

Kinasing, Ligation, and Electrophoresis. A total of 4 μg of each single-stranded DNA oligomer (Table I) was 5′-labeled with 5 μCi of [γ-^{32}P]ATP (sp act. >5000 Ci/mmol) and 5 units of T4 polynucleotide kinase in a 10-μL solution, as described before (Haran & Crothers, 1988). Each single-stranded DNA oligomer was hybridized to its complementary strand and multimerized with 2 units of T4 polynucleotide ligase at 16 °C overnight. The ligation products were analyzed on a native 8% polyacrylamide gel [monoacrylamide:bis(acrylamide) ratio 29:1 in 0.09 M Tris–borate/2 mM EDTA] at room temperature. Gels were run at 300 V until the BPB dye migrated 23 cm in a 20 cm × 38 cm × 0.8 mm gel.

Table I: Sequences Used in This Study (5′ to 3′)[a]

name	sequence
	Series I
FCT	GGGCAAAAAT
TA_5N_4	CGCTAAAAAC
$T_2A_5N_3$	CCTTAAAAAC
$T_3A_5N_3$	CTTTAAAAAC
T_4A_5N	TTTTAAAAAC
	Series II
$T_5A_5N_{11}$	GGCCTTTTTAAAAACCGGGCC
$T_5A_7N_9$	GGCCTTTTTAAAAAAACCGCC
$T_5A_9N_7$	GGCCTTTTTAAAAAAAAACCC
$T_7A_7N_7$	GGCCTTTTTTTAAAAAAACCC
	Series III
A_8N_{13}	CCGGCCAAAAAAAAAACGCGC
$A_{12}N_9$	CCGGCCAAAAAAAAAAAACGC
$A_{14}N_7$	CCGCCAAAAAAAAAAAAACG
$A_{16}N_5$	CCGCAAAAAAAAAAAAAAAC

[a] Only one strand of each duplex is shown. All deoxyribonucleotide duplexes were constructed with 2 base pairs protruding 5′ sticky ends.

Table II: T_nA_5 Series, Experiment vs Current Models[a]

	R_L − 1 (150 bp)			cooperativity quotient	
		calcd and scaled			
name	expt	wedge	junction	wedge	junction
TA_5N_4	0.93	0.93	0.93		
$T_2A_5N_3$	0.80	0.47	0.59	0.28	0.38
$T_3A_5N_2$	0.58	0.14	0.26	0.44	0.52
T_4A_5N	0.09	0.07	0.04	0.98	0.94

[a] Bend angles at the junctions [from Koo and Crothers (1988)] and AA wedge values [from Ulanovsky and Trifonov (1987)] were used to calculate circle size for the sequences, as described before (Koo & Crothers, 1988). Gel mobility anomalies (R_L − 1) were calculated from the circle size by using the equations of Koo and Crothers (1988). They were scaled to the experimental values, with TA_5N_4 being used as a reference point. The cooperativity quotient is the experimental to calculated ratio of the differences between R_L − 1 of TA_5N_4 and other sequences. Values less than 1 indicate that the observed curvature is less than predicted by model simulations.

Results and Discussion

Cooperativity in A-Tract Structure. Figure 1 shows the results of polyacrylamide gel electrophoresis on multimers of series I (Table I) and FCT (Table I and Koo et al., 1986). FCT is similar to TA_5N_4; the basic difference between these sequences is the interchange of the flanking residues at the 5′ and 3′ ends of the A tract. As can be clearly appreciated from Figure 1, TA_5N_4, $T_2A_5N_3$, and $T_3A_5N_2$ show anomalously slow migration on polyacrylamide gels, in contrast to T_4A_5N which migrates normally. The observation that TA_5N_4 and FCT show comparable retardation on polyacrylamide gels proves unequivocally that a single TA step cannot abolish the alternate A-tract structure and straighten a curved fragment. Moreover, the change to a straight helix is concentrated mainly in one step, the step from $T_3A_5N_2$ to T_4A_5N.

The sharp transition from curved DNA multimers to straight ones seems to point to a cooperative formation of an A_n-tract-specific structure ($n \geq 4$). If this is the case, then both the junction model and the wedge model should not be able to predict the observed trend in the polymer series $T_nA_5N_{5-n}$, because the current values for the bend angles at the junctions (Koo & Crothers, 1988), or for the AA wedge angles (Ulanovsky & Trifonov, 1987), were both derived without any presumptions of cooperativity. R_L − 1 is a measure of the net anomaly in gel mobility. Experimental R_L − 1 (150 bp) values for series I are presented in Table II. DNA curvature, relative to A_6, predicted for this series by the wedge

and the junction models, was calculated from the helix-axis trajectory of these sequences, as described by Koo and Crothers (1988). Quadratic dependence of $R_L - 1$ on curvature was assumed in order to calculate $R_L - 1$ values from relative curvature (Koo & Crothers, 1988). The values predicted by the models were scaled to the experimental data at a single point, with TA_5N_4 being used as a reference. We show in Table II that the values predicted for the $T_2A_5N_3$ and T_3A_5N2 multimers deviate significantly from the observed values, while the values for T_4A_5N do not. This means that neither model can predict well "partial" A-tract structures but that they are both better at predicting more fully developed ones. Furthermore, this is another indication of the minimum number, four, of adenines (or thymines) needed for establishing a functional A-tract conformation. This is demonstrated in Table II by the use of a parameter that we call the "cooperativity quotient", defined as

$$\frac{(\text{relative net anomaly})_{\text{exptl}}}{(\text{relative net anomaly})_{\text{calcd}}}$$

The relative net anomaly is calculated at each sequence by subtracting the $R_L - 1$ value for the current sequence from that of TA_5N_4, the reference point.

TA steps have long been known to possess unusual characteristics. The stacking geometry at TA steps is poor, compared to AT steps (Klug et al., 1979; Yoon et al., 1988). TA steps are less stable thermodynamically than AT steps (Breslauer et al., 1986) and are preferentially sensitive to cleavage by micrococcal nuclease and S1 nuclease (Dingwell et al., 1980; Hörz & Alterburger, 1981; Drew et al., 1985; Flick et al., 1986). Therefore, if we want to analyze the outcome of a net buildup of a T-tract structure 5′ to A_5, we have to use TA_5N_4 [and not, e.g., A_5N_5 from Koo et al. (1986)] as our point of reference for scaling the predicted $R_L - 1$ values to the experimental data. This will ensure that any peculiarity of TA steps will not interfere with our observations on T-tract structure development. The TA step is present in all members of the $T_nA_5N_{5-n}$ series; therefore, any changes in DNA conformation within this series must be ascribed to the formation of a T-tract structure at the 5′ side of an A-tract one.

The observed mobility changes in series I cannot be ascribed to helical twist changes, as T's are substituted for C or G residues. The influence of exact phasing on gel-mobility behavior is important (Koo et al., 1986). In series I, however, there is a higher content of poly(dA)·poly(dT)-like sequences in T_4A_5N than in TA_5N_4, leading to a better match of the 10-bp sequence repeat with the helical repeat in the first sequence than in the latter one.

Attempting to explain the present results in the wedge-model framework by invoking the existence of significant wedges, other than AA, cannot account well for the observed difference between the predicted values and the experimental ones. The only difference between $T_3A_5N_2$ and T_4A_5N is a CCT vs a CTT triplet. CC wedges cannot be large (Koo et al., 1986). The only other wedge is CT. This wedge, however, is present in all members of this series and is always adjacent to the T tract. Therefore, whatever magnitude and direction that this wedge may assume, it will contribute equally to all four fragments of this series and thus cannot explain the abrupt change at T_4A_5N. On the other hand, further optimization of the values for the angles at the junctions, without assumptions of cooperativity, does not result in a better fit of the experimental values to the ones predicted by the current junction model.

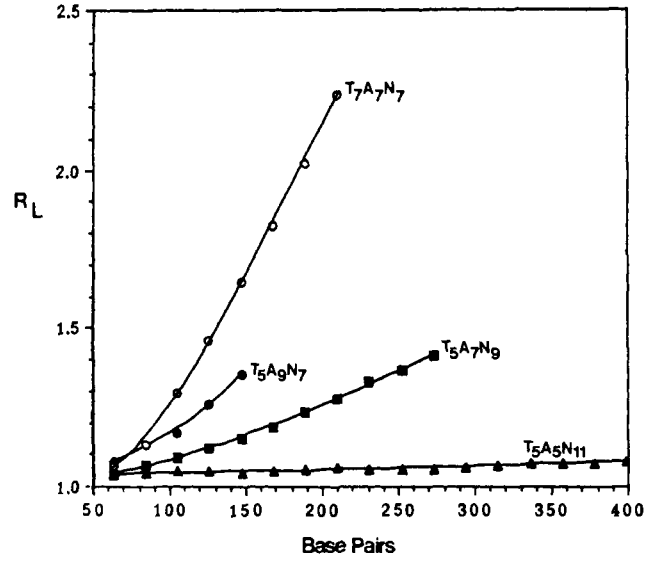

FIGURE 2: R_L values vs actual chain length for series II, $T_nA_mN_{21-n-m}$ (n = 5 or 7; m = 5, 7, or 9).

Diekmann and Kitzing (1988) have investigated cooperativity in A-tract structure. They constructed sequences where A_5 tracts are separated by one helical turn from A_n tracts (n = 0–4). When they plot the R_L (231-bp) values vs the number of A's in the second tract, they notice a nonlinear increase in R_L values in going from n = 3 to n = 4. This, they state, is not consistent with nearest-neighbor models, which should show a linear relation throughout. We have carried out model simulation for this series by both the wedge and the junction model [as described by Koo and Crothers (1988)] and scaled it to the experimental values using sequence 1, $A_5N_7AN_8$, as the reference point. We indeed observe a linear relation of R_L values to the number of A's in the second tract. Nevertheless, the straight line passes through sequence 2, $A_5N_7A_2N_7$, and sequence 3, $A_5N_6A_3N_7$, that are predicted well while sequence 4, $A_5N_6A_4N_6$, is not. If the results of Diekmann and Kitzing arise simply from a cooperative buildup of a second A-tract structure, then the straight line predicted by both models should pass through the points of sequences 1 and 4. This is because both these sequences have a fully functional A-tract structure that should be well predicted by both current models.

Very recently, Leroy et al. (1988) have shown, by NMR, that the exchange time of imino protons in A tracts is strongly dependent on A-tract size between 3 and 6 bp, consistent with a cooperative buildup of A-tract structure.

Structure of T_nA_n Blocks. The results of series I rule out the possibility that a clash in the minor groove of a single TA step, however severe, would be able to abolish the unique structure of A tracts. The structure of T_nA_n blocks may still, however, be B-like. It can be argued that the putative clash, which prevents both tracts from flipping over to the A-tract-specific structure, is not within a single TA step but between two A tracts of equal dominance and stability, positioned with opposite polarities within one T_nA_n block.

To exclude this possibility, we have constructed the series $T_nA_mN_{21-n-m}$ (n = 5 or 7; m = 5, 7, or 9; series II in Table I). Burkhoff and Tullius (1988) [see also Tullius and Burkhoff (1988)] essentially argue for another cooperative effect in A-tract structure: when one step cannot propeller twist, it hinders the neighboring steps from propeller twisting as well, and so on indefinitely along the entire length of the double helix. This "domino" effect thus destroys the structure of the whole tract. Figure 2 shows the experimental observations for

series II. The molecules, apart from $T_5A_5N_{11}$, are definitely not straight. If the domino argument is correct for series II, then $T_5A_5N_{11}$ should be straight, not because there is cancellation of junctions or wedge angles but because the alternate A-tract structure is abolished in the entire polymer. In that case, the addition of two adenines to the A tract, or two thymines to the T tract in T_nA_n blocks, might not matter much, and the effect could propagate further, yielding molecules still in a B-like conformation and straight. However, the members of series II are curved as much as predicted by the junction model. This means that a full A-tract-specific structure exists in $T_5A_7N_9$, $T_5A_9N_7$, and $T_7A_7N_7$, leading us to condlude either that a domino effect does not exist or that it is of limited length. To prove that a putative clash does not prevent even $T_5A_5N_{11}$ from being in the A-tract structure, it is sufficient to rewrite the sequence $(A_5T_5)_n$, studied by Hagerman (1987), as $(T_5A_5)_n$. The experimental R_L (150-bp) value for this sequence is 1.56, consistent with a roll junction at each TA step. According to Burkhoff and Tullius (1988) and the domino argument, this latter fragment should be a straight B-like molecule and should show normal migration on polyacrylamide gels. We conclude by stating that T_5A_5 is curved while $T_5A_5N_{11}$ is practically straight because the first fragment contains twice as many TA roll junctions as the latter one and because there are different phasing effects in the two fragments (10- vs 21-bp repeat length).

It is interesting to compare the sequence $T_5A_9N_7$ to $T_7A_7N_7$. Both sequences have B-T and A-B junctions (pure tilt junctions) 14 bp apart, but the relative positioning of the T-A junction (a pure roll junction) is different. This is reflected in the larger anomaly displayed by the $T_7A_7N_7$ sequence (Figure 2).

Structure of Elongated A Tracts and Curvature. Burkhoff and Tullius (1988) propose that curvature in A-tract structure is linked to the gradient of minor groove widths in this structure, i.e., the change from a wide, B-like groove at the 5′ end to a narrow, poly(dA)·poly(dT)-like groove at the 3′ end. The existence of a narrow minor groove in A-tract structure is undisputed (Drew & Travers, 1984; Nelson et al., 1987; Coll et al., 1987). However, it has never been shown to be the cause of global curvature. If the proposal by Burkhoff and Tullius (1988) is correct, then the further addition of A's to the tract should not affect the overall curvature, once we have elongated the A-tract structure sufficiently to reach a full poly(dA)·poly(dT)-like structure at the 3′ side of the tract. In other words, once the A tract is long enough, the bend should be located primarily at the 5′ end.

To address these issues, we have constructed a third series, A_nN_{21-n} (n = 8–16; series III in Table I). We have evidence from NMR measurements that the structure of A tracts continues to change until there are about seven bp in the tract, but stops changing thereafter (Nadeau & Crothers, 1989). The first eight bp in each polymer of series III are always in phase with the helical screw when multimerized. Therefore, if the above argument is correct, the identity of the residues beyond the first eight A's should not matter, and the whole series should exhibit the same behavior on polyacrylamide gels.

In Figure 3 we present the gel-migration behavior of series III. As expected from the junction model, the members of series III do not show the same amount of migration anomaly on polyacrylamide gels. A_8N_{13} and $A_{16}N_5$ are the most retarded polymers, and $A_{12}N_9$ is the least, consistent with the prediction by the junction model. This rules out the possibility that the mere action of closing down a minor groove can lead to global curvature. Furthermore, it shows that both junctions

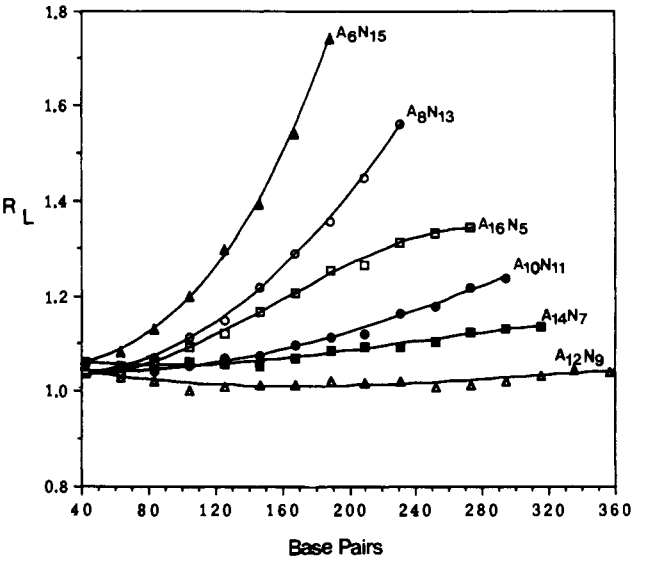

FIGURE 3: R_L values vs actual chain length for series III, A_nN_{21-n} (n = 8–16), and A_6N_{15} [the same sequence as $A_6 - {}^1/_2$ from Koo and Crothers (1988)].

are important for curvature, even when the full poly(dA)·poly(dT) character of the A-tract structure is allowed to develop. Our NMR data (Nadeau & Crothers, 1989) seem to indicate that the base pairs in the A-tract structure are negatively tilted with respect to the global helix axis. This will cause the narrowing of the minor groove. However, it is the tilting of the base pairs that causes the global bending, when A tracts are phased with adjacent B-DNA regions.

Olson's Nearest-Neighbor Model. The observations presented here argue against models based solely on nearest-neighbor interactions. Furthermore, our data are inconsistent with the nearest-neighbor curvature model of Olson and colleagues (Srinivasan et al., 1987; Maroun & Olson, 1988). According to this model, the global curvature in the double helix is generated by wedges, produced by asymmetric preferences of certain dinucleotides, as mentioned above. Qualitatively, two sequences with the same number of asymmetric base-pair steps, positioned alike, should have comparable mobilities on polyacrylamide gels. The sequence A_8C_2 has two such steps, AC and CC, and R_L (150 bp) = 2.21 (Koo et al., 1986). T_4A_5C, from the present study, has two asymmetric steps as well, AC and CT, and R_L (150 bp) = 1.09 (Table II). The small difference in the energy diagrams of CC and CT steps (Srinivasan et al., 1987) cannot account for a 2-fold difference in R_L and 4-fold difference in curvature. Furthermore, $T_3A_5C_2$ of the present study has the same two steps, AC and CC, as A_8C_2 but in addition has an adjacent asymmetric CT step. Nevertheless, $T_3A_5C_2$ has R_L (150 bp) of only 1.58. We can also compare $T_3A_5C_2$ [R_L (150 bp) = 1.58] to $T_4A_4N_2$ [R_L (150 bp) = 1.05; Hagerman, 1986]. Both sequences have three asymmetric steps, AC, CC, and CT in $T_3A_5N_2$ and AG, GC, and CT in $T_4A_4N_2$. In the energy diagrams of Srinivasan et al. (1987) the pattern for AC is very similar to that of AG, and CC to that of GC, but their R_L (150-bp) values are wide apart. The neglect of long-range interactions, which for runs of A's seems to be crucial, may account for the failure of this model.

Additional bends may be located within G·C-rich regions, but they are not the prime manifestation of "A-tract curvature". They may, however, modulate the extent of curvature by ~15%, as has been observed when A tracts are flanked by different residues (Koo et al., 1986). Moreover, variation of wedge angles in the G·C-rich regions may be

responsible for the failure of the junction model to predict simultaneously the properties of $A_5N_7AN_8$ and $A_5N_6A_4N_6$ (Diekmann & Kitzing, 1988).

CONCLUSIONS

From the present study we draw the following conclusions:

(1) The implication of series I and II is that two non-B-DNA helices, an A-tract structure and a T-tract structure, which are of opposite polarity, can be found in T_nA_n blocks ($n \geq 4$). The conformation of TA steps does not interfere with the construction of the T-tract structure and does not prevent the two altered helices from coexisting. The two structures may roll at the TA step in order to relieve a clash there, but this will not affect the conformation of the rest of the two tracts. An alternate structure, different form B-DNA, still exists within the components of T_nA_n blocks. TA steps are evidently different from AT, but it does not necessarily follow that TA steps destroy A-tract structures. It may be, however, that AT steps do add stabilization to short A tracts, such that $A_2T_2N_6$ multimers can already adopt the A-tract structure, as shown recently by Leroy et al. (1988).

(2) We have shown here that, regardless of the exact details of A-tract structure, the conformation of AA steps is highly context dependent. AA steps do not assume the same conformation in long runs of A's as they do in isolated AA steps. Therefore, the assumption of all nearest-neighbor models, that global bending is an additive phenomenon of local effects, is invalid.

(3) The extent of curvature is determined by the relative positioning of A-tract structure regions and B-DNA ones. Both the 5′ junction (B to A or T) and the 3′ junction (A or T to B) are important, as evidenced by series III, even when the A-tract structure is elongated sufficiently to acquire a full poly(dA)·poly(dT)-like character at the 3′ end of the tract. Furthermore, as demonstrated by series II, the TA junction can strongly modulate the extent of bending by the 5′ and the 3′ junctions.

In theory the curvature could still be located either within the A-tract structure or within the G·C region. One cannot at present rule out curvilinearity of A-tract or B-DNA regions. The relation between the crystal structure of short A tracts (Nelson et al., 1987; Coll et al., 1987) and curved A-tract structure in solution is still uncertain. Distamycin, a drug known to abolish bends in DNA double helices (Wu & Crothers, 1984), binds to the propeller-twisted A-tract structure without substantial changes to this structure (Coll et al., 1987). On the other hand, we know that B-DNA base-pair steps can assume wedgelike conformations, by roll and tilt motions, as evidenced by the many single-crystal X-ray structures determined to date [reviewed by Shakked and Rabinovich (1986) and Haran and Shakked (1988)]. However, even if we assume that the bends are located either within the A-tract structure, or within the G·C-rich region, the origin of the curvature of A-tract-containing sequences is still the cooperative non-B-DNA structure of the A tracts and its interaction with adjacent B-DNA regions.

ACKNOWLEDGMENTS

We thank Grace Sun for technical assistance with oligonucleotide synthesis. We also thank Levy Ulanovsky for helpful discussions and James Nadeau and Jacqueline Drak for a critical reading of the manuscript.

REFERENCES

Breslauer, K. J., Frank, R., Blöcher, H., & Marky, L. A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3746–3750.

Burkhoff, A. M., & Tullius, T. D. (1987) *Cell 48*, 935–943.

Burkhoff, A. M., & Tullius, T. D. (1988) *Nature 331*, 455–457.

Calladine, C. R., Drew, H. R., & McCall, M. J. (1988) *J. Mol. Biol. 201*, 127–137.

Coll, M., Frederick, C. A., Wang, A. H.-J., & Rich, A. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8385–8389.

Diekmann, S., & Wang, J. C. (1985) *J. Mol. Biol. 186*, 1–11.

Diekmann, S., & von Kitzing, E. (1988) in *Structure and Expression. Vol. 3: DNA Bending and Curvature* (Olson, W. K., Sarma, M. H., Sarma, R. H., & Sandaralingam, M., Eds.) pp 57–67, Adenine Press, Guilderland, NY.

Dingwell, C., Lomonossoff, G. P., & Laskey, R. A. (1981) *Nucleic Acids Res. 9*, 2659–2673.

Drew, H. R., & Travers, A. A. (1984) *Cell 37*, 491–502.

Drew, H. R., Weeks, J. R., & Travers, A. A. (1985) *EMBO J. 4*, 1025–1032.

Flick, J. T., Eissenberg, J. C., & Elgin, S. C. R. (1986) *J. Mol. Biol. 190*, 619–633.

Fratini, A. V., Kopka, M. L., Drew, H. R., & Dickerson, R. E. (1982) *J. Biol. Chem. 257*, 14686–14707.

Griffith, J., Bleyman, M., Rauch, C. A., Kitchin, P. A., & Englund, P. T. (1986) *Cell 46*, 717–724.

Hagerman, P. J. (1986) *Nature 321*, 449–450.

Hagerman, P. J. (1987) in *Unusual DNA Structures* (Wells, R. D., & Harvey, S. C., Eds.) pp 225–236, Springer Verlag.

Haran, T. E., & Crothers, D. M. (1988) *Biochemistry 27*, 6967–6971.

Haran, T. E., & Shakked, Z. (1988) *J. Mol. Struct. (Theochem.) 176*, 367–391.

Hörz, W., & Altburger, W. (1981) *Nucl. Acids Res. 9*, 2643–2658.

Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z., & Steitz, T. A. (1979) *J. Mol. Biol. 131*, 669–680.

Koo, H.-S., Woo, H.-M., & Crothers, D. M. (1986) *Nature 320*, 501–506.

Leroy, J.-L., Charretier, E., Kochoyan, M., & Gueron, M. (1988) *Biochemistry 27*, 8894–8898.

Marini, J. C., Levene, S. D., Crothers, D. M., & Englund, P. T. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7664–7668.

Maroun, R. C., & Olson, W. K. (1988) *Biopolymers 27*, 561–584.

Nadeau, J. G., & Crothers, D. M. (1989) *Proc. Natl. Acad. Sci. U.S.A.* (in press).

Nelson, H. C. M., Finch, J. T., Luisi, B. F., & Klug, A. (1987) *Nature 330*, 221–226.

Shakked, Z., & Rabinovich, D. (1986) *Prog. Biophys. Mol. Biol. 47*, 159–195.

Srinivasan, A. R., Torres, R., Clark, W., & Olson, W. K. (1987) *J. Biomol. Struct. Dyn. 5*, 459–496.

Trifonov, E. N., & Sussman, J. L. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3816–3820.

Tullius, T. D., & Burkhoff, A. M. (1988) in *Structure and Expression. Vol. 3: DNA Bending and Curvature* (Olson, W. K., Sarma, M. H., Sarma, R. H., & Sandaralingam, M., Eds.) pp 77–85, Adenine Press, Guilderland, NY.

Ulanovsky, L. E., & Trifonov, E. N. (1987) *Nature 326*, 720–722.

Ulanovsky, L. E., Bodner, M., Trifonov, E. N., & Choder, M. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 862–866.

Wu, H.-M., & Crothers, D. M. (1984) *Nature 308*, 509–513.

Yoon, C., Privè, G. G., Goodsell, D. S., & Dickerson, R. E. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 6332–6336.