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New Insights into the Structure of A_n Tracts and B'-B' Bends in DNA[†]

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ABSTRACT: Energy calculations suggest that the currently available NOE distance constraints for A_n tracts in DNA are incapable of distinguishing between structures with a narrowed minor groove arising from a large propeller twist with a small inclination or from a small propeller twist with a large negative inclination. Furthermore, analysis of published data, together with energy estimations, strongly argue against bifurcated hydrogen bonding between A and T residues being the cause of the anomalous structural properties of A_n tracts. A conformational analysis of the B'-B' junction has been performed in which a single variable base pair has been inserted between two regions of B' structure. We have calculated low-energy structures for A_nGA_n , A_nCA_n , A_nTA_n , A_nCT_n , and T_nCA_n duplexes, where the A_n and T_n tracts were fixed in the anomalous B' conformation. Upon optimization, all these structures were found to contain a pronounced roll-like bending into the major groove at the site of the insertion. The important factors in the formation of these B'-B' bends are the destruction of the B' conformation and the concomitant widening of the minor groove at the junction region in order to reduce minor groove interstrand base clashes and improve interstrand stacking energy. If the B' conformation has strong negative inclination, the improved intrastrand stacking energy also contributes to the bending. In calculations of duplexes with A_n and T_n tracts in the B conformation instead of B', the bending disappears.

DNA bends, discovered several years ago (Marini et al., 1982; Hagerman, 1984; Wu & Crothers, 1984; Griffith et al., 1986; Ulanovsky et al., 1986), continue to attract the attention of both theorists and experimentalists. It has been shown recently that DNA bends are implicated in several biological functions, and various possible molecular mechanisms for bending have been discussed (Trifonov, 1985; Koo et al., 1986; Diekmann, 1987; Olson et al., 1988; Hagerman, 1990; Crothers et al., 1990). One of the models that accounts for the experimental data quite well is the so-called "distributed-junction" model proposed earlier by Chuprina and Abagyan (1988a), in which bending is distributed over several base pairs. An independent model with a similar direction of bending was also proposed by Koo and Crothers (1988). The two models have much in common, though they were obtained in different ways: the Koo-Crothers model is based on gel electrophoretic data, whereas the Chuprina-Abagyan model arose largely from molecular mechanics studies. One of the important elements of the Chuprina-Abagyan model is the use of a B'-type A_n tract structure that agrees well with available NMR (Behling & Kearns, 1986; Kintanar et al., 1987; Katahira et al., 1988; Celda et al., 1989; Nadeau & Crothers, 1989) and X-ray (Alexeev et al., 1987; Coll et al., 1987; Nelson et al., 1987; Park et al., 1987; DiGabriele et al., 1989) data. This model for overall bending considers bends

induced by the B-B' junction as well as those resulting from junctions between A_n and T_n tracts, i.e., B'-B' junctions (designated B'-B' bends). From these calculations, the magnitude of B'-B' bends should be significantly larger than that of B-B' bends. Our earlier molecular mechanics studies (Chuprina & Abagyan, 1988a) also indicate that T_nA_m , A_nCGT_m , A_nGCT_m , T_nGCA_m , and T_nCGA_m sequences have a pronounced roll-like bending into the major groove in the -TTAA-, -ANNT-, and -TNNA- regions, whereas the A_nT_m sequence is unbent (or bent very little). These results provide an explanation for the dramatically different electrophoretic mobility of $[G_A T_A C]_n$ versus $[G_T A_A C]_n$ multimers observed by Hagerman (1986). Indeed, according to our calculations, bends should exist in the $[G_A T_A C]_n$ multimers at both ends of the A_4T_4 region but not in the middle. However, in the $[G_T A_A C]_n$ multimers there is an additional bend at the central junction TA that counteracts the effects of the peripheral bends, resulting in little or no overall curvature. We have also proposed (Chuprina & Abagyan, 1988a) that insertion into an A_nT_m tract of any random base pair that produces a step other than AA or AT will result in a B'-B' bend into the major groove in the region of this insertion.

The availability of new experimental data has now prompted us to reexamine the conformational possibilities of the oligo-(dA)·oligo(dT) structure. We now show that the bifurcated hydrogen bonds found in the major groove of the A_n tracts (Nelson et al., 1987) are not the main determinants of either the structural features or the anomalous properties of A_n tracts. Our conformational analysis suggests that there are still ambiguities in interpreting A_n tract NOE data in terms of helical

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parameters, in particular inclination and propeller twist. Molecular mechanics studies have been performed for DNA fragments A_nCA_m , A_nGA_m , A_nTA_m , A_nCT_m , and T_nCA_m . The results lead to the ability to interpret and predict bends in a number of sequences containing B'-B' junctions.

METHODS

Low-energy conformations were determined as a sum of atom-atom van der Waals and electrostatic interactions, torsional energies of rotations around single bonds of the sugar-phosphate backbone, and energies of the internal bond angles of deoxyribose and the C3'-O3'-P angle. Other bond angles and bond lengths in DNA were assumed to be constant with the same values published previously (Chuprina et al., 1981; Chuprina, 1987). The parameters for the potential functions were taken from Zhurkin et al. (1980) for nucleic acids and from Poltev et al. (1984) for water-water and water-DNA interactions. Calculations were performed by assuming that the dielectric constant ϵ was 4 and the phosphate groups were completely neutralized, as described earlier (Chuprina, 1987), unless noted otherwise. The hydrogens of the thymine CH₃ group were oriented as in Chuprina (1987). Initial deoxyribose conformations were placed in the C2'-endo region, and all backbone torsion angles are in the BI type conformation (Dickerson et al., 1985). Energy minimization was performed in the space of independent variables, which in the case of poly(dA)·poly(dT) were the following: (1) the parameters characterizing the position of the base pair relative to the helix axis, namely, tip (θ), inclination (η), y displacement (d_y), and x displacement (d_x); (2) the helical parameters twist (Ω) and rise (D_z); (3) two torsion angles χ , defining the glycosidic base-sugar rotamer for each strand; (4) the eight angles determining the conformations of sugar rings in both complementary residues (two torsion and two bond angles for each sugar); (5) either simple propeller twist (ω) or the six parameters characterizing the mutual position of the two bases in the pair, namely, stagger (S_z), stretch (S_y), shear (S_x), opening (σ), propeller twist (ω), and buckle (k). All these definitions, except one, were used as defined in the Cambridge Convention (Dickerson, 1989). It should be noted that the sign of ω we use is in agreement with the more common literature practice for reasons of convenience; this usage is opposite in sign to that of the Cambridge Convention recommendations. For the poly(dA)·poly(dT) complex with the spine of hydration, 12 more degrees of freedom were added as variables for water molecules. These variables, and the calculating procedure for the energy of the DNA complex with water, are the same as described previously (Chuprina, 1987). In calculations, the twist angle was fixed at $\Omega = 36^\circ$ to produce 10 bp per turn.

The following method for determining the bending of helix axes in DNA fragments was used. The conformational parameters of the two outer nucleoside pairs of a trimer were fixed. Their values were the same as for the corresponding regular poly(dA)·poly(dT) conformations in Table I. The bending angle β is defined as the angle between helical axes K_1 and K_2 of the rigid flanking poly(dA)·poly(dT) structures (see Figure 2 and legend). The remaining independent parameters of the trimers were allowed to vary as described earlier (Chuprina & Abagyan, 1988a). The only difference from the earlier study was the introduction of greater base pair flexibility; when the outer residues were placed in conformation II, all six degrees of freedom (not just propeller twist) were allowed for the inner pair in the present study. It should be noted that calculations on a trimer with fixed outer nucleoside pairs are equivalent to calculations of DNA fragments con-

Table I: Conformational Parameters for the Poly(dA)·Poly(dT) Helices Used as Flanking Segments^a

no.	L	d_x	η	ω	D_z	Ω	S_z
I	9.6	1.01	-2.3	20.6	3.21	36.0	0
II	9.6	1.1	~0	21.5	3.09	36.0	0.5
III	9.4	0.89	-7.8	10.8	3.35	36.0	0
IV	11.9	2.18	-0.3	1.0	3.41	33.2	0

^a Distances are in Å, and angles are in degrees. L is the minor groove width (the distance between the nearest phosphorus atoms of the antiparallel chains); d_x is the shift of the base pair from the helix axis at right angles to the long axis of the base pair; ω is the propeller twist with the opposite sign to that suggested in the Cambridge convention (Dickerson, 1989), i.e., nearer base rotated clockwise with respect to the more distant base; D_z is the rise distance between adjacent base pairs along the helix axis; Ω is the twist angle; S_z is the stagger displacement along the helix axis between the bases of a single base pair; η is the inclination with respect to the helix axis. The dihedral backbone angles for the fixed flanking segments in conformations I-IV are in the following ranges: α (-58 to -70), β (177-185), γ (53-60), δ (122-133), ϵ (179-183), and ζ (-101 to -104), as in P⁺O5'- β C5'- γ C4'- δ C3'-O3'-P. Structures I, III, and IV presented here were taken from Chuprina and Abagyan (1988a). Structure II is closely similar to those published in Lipanov and Chuprina (1987). Structure II was obtained in the present work.

sisting of two fixed regular double-helical segments with a flexible base pair connector between them (see Results and Discussion).

Our approach to finding the low-energy conformation involves a facilitated procedure for energy minimization. This approach uses fixed lengths for all bonds and rigid base templates, and all exocyclic bond angles, except C3'-O3'-P, are fixed (Chuprina et al., 1981); all torsion angles and all intrasugar bond angles (as well as C3'-O3'-P) are given complete freedom. This approach is more reliable than atom-by-atom energy minimization in finding global minima because of the lack of large energy barriers arising from flexible bond lengths and is obviously faster and more efficient as a result of the reduction in the number of independent parameters. Such an approach can only find the region of the true global minimum if given a reasonable starting point. In the present studies we used starting points within the regions previously demarcated by exhaustive calculations on homopolymer B-like conformations in which the reduced number of degrees of freedom permitted the scanning of all structural space (Zhurkin et al., 1978; Chuprina et al., 1981). To further ensure that the resulting minimum is not merely a local minimum, we finally scanned the most important parameters, one at a time, while minimizing all the other parameters. Furthermore, several independent starting points within the allowed range of B-like conformations were used to initiate the calculations.

RESULTS AND DISCUSSION

On the Structure of A_n Tracts in Solution. It is known that the A_n tract in solution has 10.0 bp per turn and a narrowed minor groove compared to B-form DNA (Strauss et al., 1981; Peck & Wang, 1981; Rhodes & Klug, 1981; Drew & Travers, 1984; Behling & Kearns, 1986; Kintanar et al., 1987; Lipanov & Chuprina, 1987; Burkhoff & Tullius, 1988; Katahira et al., 1988; Celda et al., 1989; Nadeau & Crothers, 1989). On the basis of NOE-measured interproton distances and coupling analysis, it has been concluded that base pairs in the A/T tract are characterized by a strong propeller twist (Katahira et al., 1988; Celda et al., 1989). In particular, it was shown by Katahira et al. (1988), and corroborated by Nadeau and Crothers (1989), that in runs of six or more A residues the average ratio of the intensities for the two NOEs between the T N3H and the H2 of its own adenine compared to the H2

Table II: Interproton Distances in the Minor Groove of dA_ndT_n Tracts^a

structure	<i>a</i>	<i>d</i>	<i>c/b</i>	(<i>c/b</i>) ^b
III from Table I	3.64	3.89	0.87	0.43
II from Table I	3.74	3.92	0.82	0.3
NMR, A ₇ tract ^b	3.5	3.8	0.89	0.5
NMR, A ₅ tract ^b	3.8	4.0	0.88	0.46

^aDistances are in Å; *a-d* are the distances between the H2 of adenine and (a) H1' of its 3'-neighbor of its complementary thymine, (b) H3 of the 3'-neighbor of its complementary thymine, (c) H3 of its own complementary thymine, and (d) H1' of its 3'-neighbor adenine, respectively. *c/b* is the ratio of distances, and (*c/b*)^b is the inverse ratio of NOE intensities; *a-d* designations are as in Nadeau and Crothers (1989). ^bData taken from Nadeau and Crothers (1989).

of the 5'-neighboring adenine is about 1.0/0.4, corresponding to an H2 distance ratio of 0.86/1.0 to the T N3H. In interpreting this result, Katahira et al. (1988) compared three structures, classical B-form DNA (Arnott & Hukins, 1972), the heteronomous poly(dA)·poly(dT) model structure (Arnott et al., 1983), and the central part of the Dickerson dodecamer (Dickerson et al., 1985), and came to the conclusion that the observed NOE intensity ratio came closest to that expected for the structure of the A/T tract in the Dickerson dodecamer, which has an average propeller twist of ca. 17°. They therefore assumed that the propeller twist of their A₆ tract in solution was somewhat larger than 17°.

However, in the earlier structure proposed by Lipanov and Chuprina (1987) for an A_n duplex, the expected NOE intensity ratio from the corresponding distances would be 1.0/0.43 (in good agreement with the measured values) despite the fact that the structure actually has a smaller propeller twist (~12°) and an inclination of ca. -7°. A similar conclusion has also been made by Nadeau and Crothers (1989); i.e., this NOE ratio can also be achieved in low-energy structures with a propeller twist smaller than 12°, provided negative inclination is also introduced. Thus, NOE measurements of the N3H to H2 intensities do not necessarily indicate the existence of a large propeller twist.

On the other hand, our present calculations performed for poly(dA)·poly(dT) with a fixed helical twist of 36° and a spine of hydration in the minor groove (Drew & Dickerson, 1981) give a possible low-energy conformation (slightly higher energy than the optimal one) with an inclination value near zero and a propeller twist value of ~20°, in which the corresponding ratio of NOE intensities would be 1.0/0.3, i.e., with relative distances from the N3H to the complement/neighbor H2 of 0.82/1.0 (see Figure 1 and Tables I and II). It is worth noting that these values emerge from the molecular mechanics calculations without any proton-proton distance constraints. Examination of the experimental data reveals that this is well within the experimental error of the distance measurement, and furthermore, the experimentally observed ratio appears to vary from ca. 0.3 to ca. 0.6 for different positions in the A₆T₆ tract (Katahira et al., 1988). The calculated structure thus satisfies the relevant distance ratio rather well (Behling & Kearns, 1986; Katahira et al., 1988; Nadeau & Crothers, 1989), though slightly less well than the poly(dA)·poly(dT) structure, with an inclination of ~7° and a propeller twist of ~12° (Lipanov & Chuprina, 1987) (see Table II). Thus, low-energy structures of the A_n tract with a narrowed minor groove and either a large propeller twist (~20°) combined with a small inclination (~0°) or a small propeller twist (~12° and less) combined with a greater negative inclination (~-7° or more) both satisfy all the available NMR data quite well—see also Lipanov and Chuprina (1987) and Chuprina (1987). We therefore conclude that measurement of the T-H3

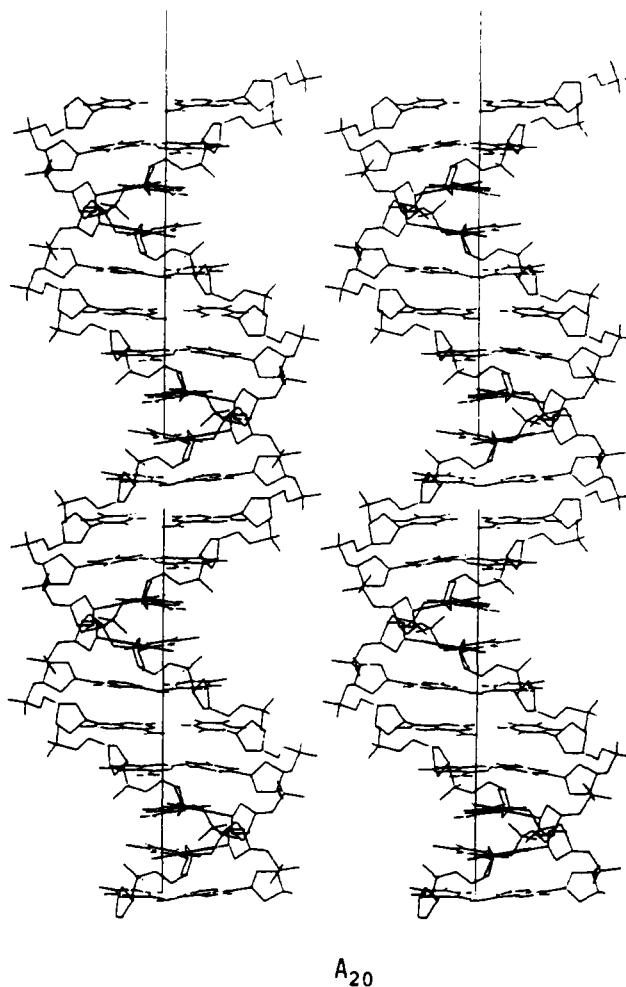


FIGURE 1: Stereoview of poly(dA)·poly(dT) with distances of 3.2 Å between the A N6 and the T O4 of the neighboring pair (conformation II from Table I). The mutual stagger shift of thymine relative to adenine is *S*_z = 0.5 Å along the helix axis. The coordinates are available on request.

NOE ratio (or relative distance) to the complementary AH2 versus the neighboring AH2 is insufficient to characterize or define the propeller twist angle; neither is it sufficient to determine the inclination angle. Furthermore, the additional intrastrand (*n*)H2-(*n* + 1)H1' and interstrand (*n*)H2 to (*m* + 1)H1' distances [where *n* and *m* are complementary and (*n* + 1) and (*m* + 1) are their 3'-neighbors] do not resolve this ambiguity (see Table II).

On Bifurcated Hydrogen Bonds in the Major Groove of the A_n Tract. In the previously calculated poly(dA)·poly(dT) structure with an inclination of ~-7° and a propeller twist of ~12° (Lipanov & Chuprina, 1987), the cross-strand distance between adenine N6 and the O4 of the thymine complementary to its 3'-neighbor is ~4.0 Å and therefore does not satisfy the criterion for a cross-strand bifurcated hydrogen bond as determined for oligo(A) tracts in the crystal state (Coll et al., 1987; Nelson et al., 1987). In the structure shown in Figure 1 this distance was now constrained to 3.2 Å in order to enforce a bifurcated hydrogen bond. However, if this distance is not fixed, the energy optimization for poly(dA)·poly(dT) containing a spine of hydration in the minor groove produces a structure with N6-O4 distances larger than 3.5 Å, and the energy improves over that of the structure with these cross-strand distances constrained to 3.2 Å—but only by a rather small amount (0.3 kcal/mol). Thus the geometry of bifurcated hydrogen bonds is not observed at the energy optimum, although it cannot be completely ruled out since the

molecular mechanics results depend on the particular values for the potentials, charges, and parametrization.

The available data suggest that the geometry required for bifurcated hydrogen bonding does not cause, nor does it stabilize, the narrowing of the minor groove. When we optimized poly(dA)·poly(dT) without a spine of hydration, but with a fixed cross-strand distance that ensured bifurcated hydrogen bonds, we obtained perfectly acceptable low-energy structures with a wide minor groove. A similar effect was also observed directly in crystals (Prive et al., 1987); in the decamer

1-CCAAGATTGT-10
20-GGTTAGAAC-11

the distance between N6 of A3 and O4 of T17 (and also between A13 N6 and the T7 O4) is 3.0 Å, which could be interpreted as a bifurcated hydrogen bond. However, the propeller twist of the pair A₃T₁₈ is only 11° and the minor groove width is 13 Å. It should also be mentioned that in the crystal structures of CGCAAATTTGCG, CGCAAAAAGCG, and CGCAAAAATGCG (Coll et al., 1987; Nelson et al., 1987; DiGabriele et al., 1989), where such bifurcated bonds were first proposed, most N6A–O4T cross-distances are in the range 3.1–3.4 Å, i.e., are rather long. In the complex of the oligonucleotide CGCAAATTTGCG with distamycin, these bonds are shorter (Coll et al., 1987). The same is also true for the complex of CGCGAATTCGCG with netropsin (Kopka et al., 1985), where the bifurcated hydrogen bond geometry is a secondary effect of DNA interaction with ligands. As noted previously (Chuprina & Abagyan, 1988b), experiments in which inosine replaces adenine in A_n tracts (Leslie et al., 1980; Diekmann et al., 1987) also shed light on the role of bifurcated bonds in the major groove of dA_n·dT_n duplexes. Even in (AI)_n tracts, in which no bifurcated bonds can be formed, the anomalous A_n-tract properties are still observed (Leslie et al., 1980; Diekmann et al., 1987; Chuprina & Abagyan, 1988b; Leroy et al., 1988). Furthermore, it is becoming clear that bifurcated bonds are rather common features of “nonanomalous” sequences in crystals (Heinemann & Alings, 1989).

Taken together, these results indicate that bifurcated hydrogen bonds are not themselves the determinants of, or even a requirement for, the anomalous properties and the atypical structure of the A_n tract in which the minor groove is narrowed. In contrast, the Dickerson spine of hydration in the minor groove of the A_n tract appears to be such a cause-and-effect determinant, as has been previously proposed (Chuprina, 1985); it was suggested that the curvature discovered in DNA sequences containing A_n tracts is related to stabilization of the narrow minor groove B' structure of the A_n tract by this spine of hydration and that upon disruption of the hydration spine, i.e., upon heating, the anomalous narrow minor groove structure of the oligo(A) tract (without a TpA step) converts to an ordinary B conformation with a wide minor groove and the curvature disappears, as observed experimentally at elevated temperatures (Marini et al., 1982; Koo et al., 1986; Diekmann, 1987; Olson et al., 1988). This model has been discussed in more detail elsewhere (Chuprina, 1987; Chuprina & Abagyan, 1988a). The observed requirement for four consecutive A/T residues (without a TpA step), e.g., AAAA, AAAT, AATT, ATTT, or TTTT, in order to express the anomalous properties of DNA correlates with the number of A·T pairs in A/T tracts (without a TpA step) required for the formation of the spine of hydration in the minor groove that stabilizes this anomalous conformation (Chuprina, 1987).

Low-Energy Conformations of the Sequences A_nGA_m, A_nCA_m, A_nTA_m, A_nCT_m, and T_nCA_m. In the following series

of calculations we investigate the effect of single base insertions on bending in long A_n, A_nT_n, and T_nA_n tracts. The approach involves assuming that the flanking A_n and T_n sequences have a constant B' conformation except for the four terminal residues adjacent to the insertion on either side, which are given rigid nucleoside B' conformations but flexible backbone connections to the inserted base pair which is also allowed complete conformational freedom. Given these constraints, one needs to carry out energy calculations on only the central three base pairs to investigate the junction since the residues outside the central trimer do not change energy regardless of the bending angle. Furthermore, calculations indicate that the energy between the central trimer and the fixed flanking segments changes negligibly as a function of bending angle if the phosphate groups are neutralized. To justify this computational simplification, we also included the innermost two or four base pairs of the flanking regions, with and without phosphate neutralization, in some calculations; the results demonstrate that the qualitative conclusions on bending do not change from the simpler trimer calculations. We then search for the effects of setting the bending angle β (see Methods) to various values by optimizing the trimer energy at each value of β . We then assess the extent of bending from the β value of the minimum-energy structure. It is obvious that the conclusions to be drawn from such an approach are only as good as the input assumptions described above; these assumptions are all quite reasonable, representing the most typical values for all assumed parameters, and represent the best case for investigating the qualitative effects of insertions on helix bending.

We have carried out energy calculations on the double-stranded duplexes AGA, ACA, ATA, ACT, and TCA in which the sugars and bases of the two outer base pairs were constrained to an anomalous B' conformation that exhibits a narrowed minor groove typical of A_n tracts and of poly(dA)·poly(dT). The narrow groove B' conformation can be achieved by various combinations of structural parameters, each of which was investigated in the present study. In the first set of calculations the flanking region was fixed in the B' conformation I by using the combination of parameters listed in Table I. Structures of some sequences were also calculated with the different B' conformational parameter combinations II and III (Table I). As we predicted in earlier studies (Chuprina & Abagyan, 1988a), all these structures are curved, i.e., all minimum-energy structures required nonzero β values; furthermore, a considerable “roll-like” component of bending directed into the major groove was observed (see legend to Figure 4).

What is the reason for bending in these sequences? An answer to this question follows from the data presented in Tables III–V. Locking all three base pairs of each calculated trimer in conformation I results in a straight pure B' structure that has close steric contacts in the minor groove between adjacent base pairs involving residues on opposite strands. We next subjected this structure to energy minimization with the outer four base–sugar moieties internally constrained to conformation I but allowing the four sugar–sugar linkers and the central two base–sugar residues to have conformational freedom. This produced curved structures for all five sequences, and the energy changes from straight to curved topology are summarized in Table III. In all cases the more favorable energy of the curved structure is due mainly to the van der Waals component of the *interstrand* stacking, namely, between G and T in the step ApG (CpT) and between the two purines in pyrimidine–purine steps.

Table III: Change of the Trimer Energy upon Transition from the Straight Anomalous B' Conformation I into a Curved Structure in the Process of Optimization^a

	ΔE^b	ΔE_3^c	ΔE_8^d	ΔE_{15}^e	ΔE_{26}^e	ΔE_{24}^e	ΔE_{35}^e
5'-A ₁ C ₂ A ₃ T ₆ G ₅ T ₄ -5'	-99.23	-0.1	-98.56	-0.12	-0.06	-0.64	-97.74
5'-A ₁ G ₂ A ₃ T ₆ C ₅ T ₄ -5'	-35.74	-0.05	-37.09	-0.5	-35.61	-0.22	-0.76
5'-A ₁ T ₂ A ₃ T ₆ A ₅ T ₄ -5'	-30.6	-0.15	-31.62	+0.02	+0.08	+0.02	-31.88
5'-T ₁ C ₂ A ₃ A ₆ G ₅ T ₄ -5'	-61.14	-0.16	-61.35	-0.06	-1.02	-0.5	-59.82
5'-T ₁ C ₂ T ₃ T ₆ G ₅ A ₄ -5'	-34.39	-0.27	-36.01	+0.1	+0.09	-0.52	-35.54

^aThe energy is in kcal/mol. ^b $\Delta E = E' - E$ is the difference between the optimal energy (E') of the curved trimer and the energy (E) of the trimer in the straight B' conformation I (Table I), with the terminal nucleosides fixed in conformation I. ^c ΔE_3 is the change of intrastrand stacking in the process of optimization. ^d $\Delta E_8 = \Delta E_{15} + \Delta E_{26} + \Delta E_{24} + \Delta E_{35}$ is the change of interstrand stacking. ^e ΔE_{ij} is the change of the interaction energy of bases i and j .

Table IV: Minor Groove Width, Resulting Bend Angle β , and Difference in Trimer Energy between Optimized Straight Structures with $\beta = 0$ and Optimized Structures in Which β Was Unconstrained^a

	L_1	L_2	β	ΔE_{cs}	ΔE_{st}	ΔE_{ee}
5'-ACA TGT-5'	10.0	12.7	19	-3.4	-3.1	-0.03
5'-AGA TCT-5'	9.7	11.7	16	-2.7	-2.6	-0.24
5'-ATA TAT-5'	10.0	12.5	15	-2.3	-2.4	0.06
5'-TCA AGT-5'	10.0	12.5	17	-2.6	-2.7	0.08
5'-ACT TGA-5'	10.2	12.1	17	-2.1	-2.5	0.26

^aTerminal nucleosides of all trimers were fixed in the anomalous conformation I. L_1 is the maximal minor groove width in the trimer optimized with a fixed bending angle $\beta = 0$. L_2 is the maximal minor groove width in the trimer optimized with an unconstrained bending angle β . β is the bending angle between axes K_1 and K_2 of the flanking helices—see Figure 4. $\Delta E_{cs} = E_c - E_s$, where E_s is the optimal energy of the trimer with a straight helical axis and E_c that of a trimer with a bent axis; ΔE_{st} is the total change in stacking energy; ΔE_{ee} is the change in the electrostatic part of the stacking. Distances are in Å, angles are in degrees, and energy is in kcal/mol. If calculations are performed with charged instead of neutralized phosphates, the curvature (β) increases somewhat, and the minor groove widens more in the regions AGA, ACA, ATA, ACT, and TCA due to repulsion of similarly charged phosphates.

We next asked the question, is curvature an absolute requirement for reducing the energy or are there equivalent low-energy forms with a straight topology, i.e., could other variables besides curvature permit an energetically equally acceptable straight structure? To this end we next optimized these trimers in a straight form by fixing the bending angle β at 0°. This resulted in the axes K_1 and K_2 mutually shifting along an axis approximately coincident with the long axis of the middle pair (see Figure 2); this successfully eliminates the

clash between bases in the minor groove and improves interstrand stacking. However, the *intrastrand* stacking now becomes worse compared to that in the initial straight conformation. If we now allow the bending angle, β , freedom to vary, bending into the major groove invariably occurs in the process of minimization. As seen from Table IV, the energy change is mainly due to better inter- and intrastrand stacking, and the previously poor intrastrand stacking greatly improves, regaining the excellent intrastrand energy of the starting structure. In the process the lateral displacement between axes K_1 and K_2 tends to 0 (see Figure 3).

A structure entirely in conformation I is characterized by a large propeller twist and a small inclination. In order to understand what changes would occur if other possible anomalous conformations were used in the calculations, we performed the molecular mechanics studies on some of the trimers using flanking conformations II and III. In conformation II the two complementary bases of a base pair are mutually displaced (staggered) along the helix axis ($S_z = 0.5$ Å), and in the boundary structures the distance between A-N6 and O4 of the diagonally opposite T is constrained to have a bifurcated hydrogen bond, as mentioned above. The propeller twist is large and the inclination is approximately 0°. Fixing the trimers in this conformation results in worse interstrand stacking, as occurred for conformation I, and further produces purine-purine clashing in the major groove. However, the reduced interstrand stacking as a result of major groove clashes can easily be avoided without changing the width of the minor groove, and without bending—for instance, by inclination and its associated slide. The improved interstrand stacking that results from decreasing the contacts in the minor groove is the major cause of bending. The resulting bending is somewhat smaller than that obtained with conformation I. This is due partly to the staggered mutual displacement of bases within a pair along the helix axis in conformation II (which results

Table V: Differences between Intrastrand and Interstrand Stacking Energies of Optimal Curved and Straight Conformations

	ΔE_{st}	ΔE_{12}	ΔE_{56}	ΔE_{23}	ΔE_{45}	ΔE_{15}	ΔE_{26}	ΔE_{24}	ΔE_{35}
5'-A ₁ C ₂ A ₃ T ₆ G ₅ T ₄ -5'	-3.1	0.02	-0.76	-0.30	-0.53	-0.12	-0.31	-0.08	-1.00
5'-A ₁ G ₂ A ₃ T ₆ C ₅ T ₄ -5'	-2.6	0.06	-0.38	-0.16	-0.41	0.01	-1.40	0	-0.01
5'-A ₁ T ₂ A ₃ T ₆ A ₅ T ₄ -5'	-2.4	0.23	-0.45	-0.92	-0.75	-0.24	0.14	-0.02	-0.47
5'-T ₁ C ₂ A ₃ A ₆ G ₅ T ₄ -5'	-2.7	-0.37	-0.74	-0.24	-0.4	-0.13	-0.11	0.07	-1.07
5'-A ₁ C ₂ T ₃ T ₆ G ₅ A ₄ -5'	-2.5	-0.22	-0.21	-0.73	-0.17	-0.13	-0.05	-0.06	-0.95

^aConformations are the same as in Table IV. ΔE_{st} is the change of full stacking (see notes to Table IV). ΔE_{ij} is the change of the interaction energy between bases i and j .

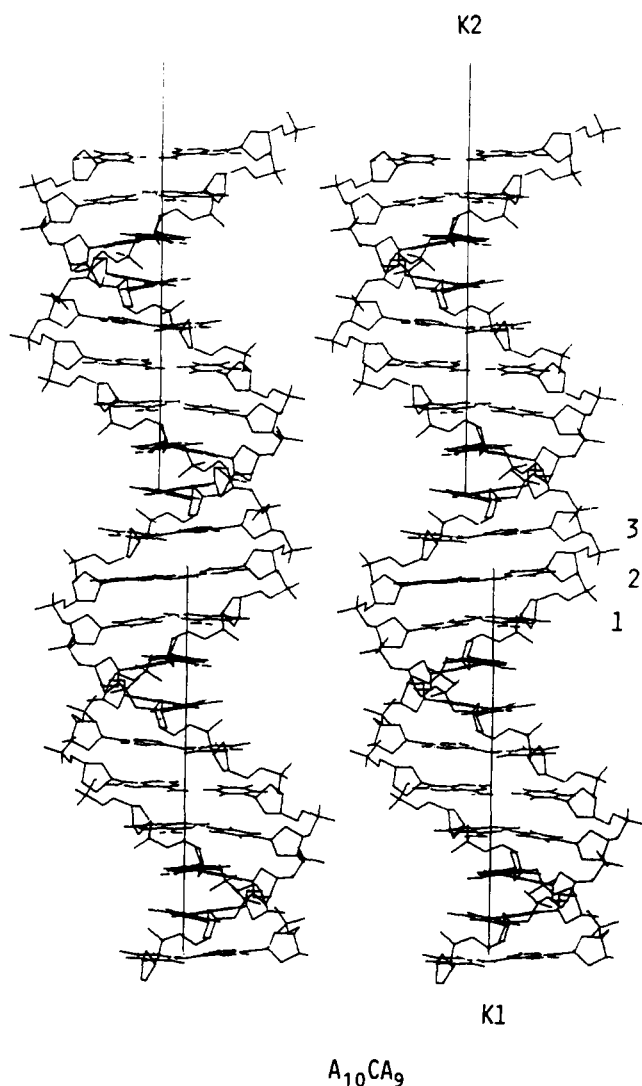


FIGURE 2: Stereoview of $A_{10}CA_9$ duplex obtained by energy optimization of the ACA fragment with fixed bending angle $\beta = 0^\circ$ (the base pair numbering is as follows: 1-A·T, 2-C·G, 3-A·T). The outer nucleoside pairs 1 and 3 are fixed in conformation I. Regular flanking helices have the same conformation. K_1 and K_2 are the axes of these helices. The mutual shift between axes K_1 and K_2 is greater than 2 Å.

in a reduction in the clash between bases on opposite chains) and partly to the use of a more flexible inner base pair for these calculations in which six degrees of freedom were allowed instead of a single propeller twist value.

Conformation III is characterized by a small propeller twist and a considerable negative inclination. The structures calculated with this boundary conformation have values and direction of the bending angle similar to those obtained with conformation I (see Table IV and legend to Figure 4). In this case, in contrast to those described above, the energy improvement is due not only to the interstrand but also to intrastrand stacking. The calculations indicate that the intrastrand stacking in a straight nonoptimized conformation III structure is worse than in a nonoptimized conformation I structure; however, upon optimization the intrastrand stacking improves, reaching the quite respectable intrastrand values of conformation I. The main reasons for the reduced intrastrand stacking in structures with negative inclination appear to be the pronounced roll of bases into the minor groove, combined with slide.

In summary, it appears that the main cause of bending in these sequences with B' boundary conformations is improve-

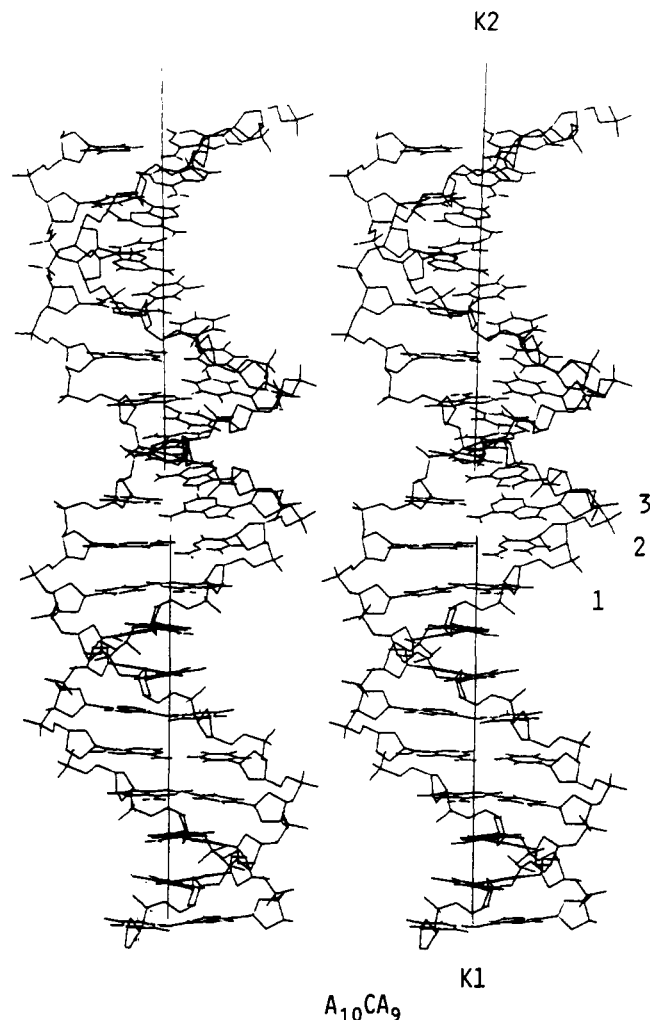


FIGURE 3: Stereoview of $A_{10}CA_9$ duplex with optimal energy after minimizing all parameters including β ; the bend angle β is 18° . All designations are the same as in Figure 2. The minor groove widens to 12 Å in the central region.

ment in the stacking energy, mainly the interstrand interaction of a base with its complement's 3'-neighbor. Increased intrastrand stacking would also make an additional stability contribution if the B' conformation of the A/T tract has strong negative inclination.

Minor Groove Width and Bending. Earlier we came to the conclusion that T_nA_m , A_nGCT_m , A_nCGT_m , T_nCGA_m , and T_nGCA_m tracts have a pronounced roll-like bending into the major groove in the central region junction (Chuprina & Abagyan, 1988a). In the region of bending the narrow flanking minor groove widens to ~ 12 Å. The causes of the bending in such sequences are the same as those discussed in the previous section for the current trimers. In contrast, the minor groove of an A_nT_m tract does not widen at the center due to the stabilizing effect of the spine of hydration, and the ApT step in this tract has no obvious bending (Chuprina & Abagyan, 1988a). Thus we have calculated all possible combinations of junctions between A_n and/or T_m tracts joined directly to each other or separated by one or two base pairs. Only the A_nT_m tract ($n + m \geq 4$) maintains an ~ 9 -Å narrowed minor groove as shown experimentally (mentioned above) and by energy calculations (Chuprina & Abagyan, 1988a). Thus, the molecular mechanics calculations indicate that insertion into an A_nT_m tract of any base that produces a step other than ApA (TpT) or ApT should result in widening of the minor groove at the point of insertion. This result correlates very well with experimental observations from OH

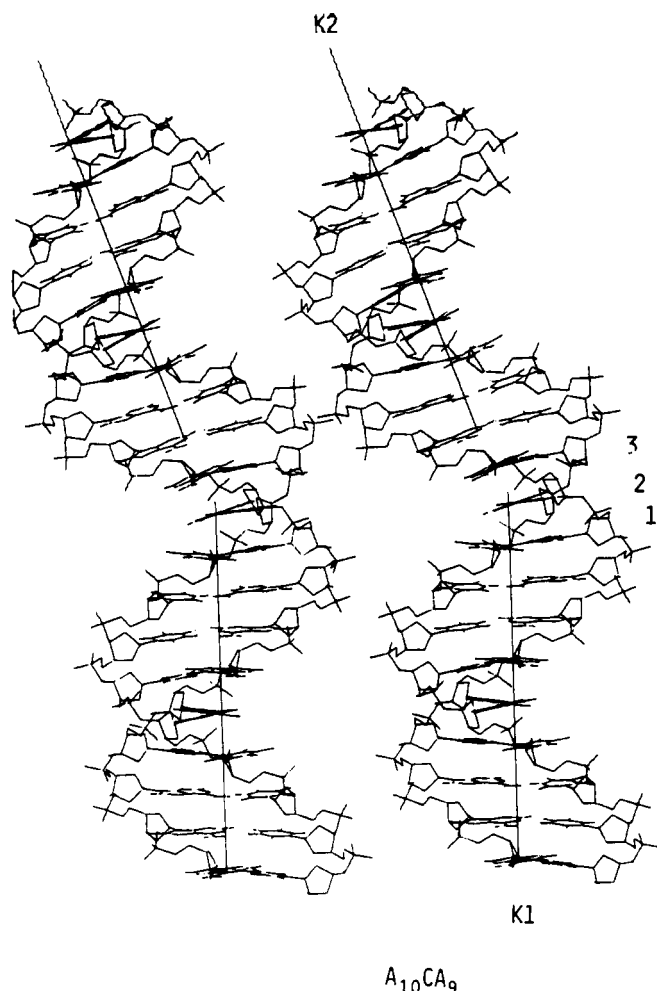


FIGURE 4: Stereoview of the same structure as in Figure 3 but turned 90° about K_1 ; β is 18° and α (Chuprina & Abagyan, 1988a), the direction of bending, is 88.2°, so that the projection of the K_2 axis on the plane perpendicular to axis K_1 almost coincides with the direction of the short axis of pair 2. For other trimers, the resulting α is within $\pm 10^\circ$.

radical cleavage (Burkhoff & Tullius, 1988), imino proton exchange (Leroy et al., 1988), and solution structure determination (Reid et al., to be submitted for publication).

It follows from our calculations that there is an obligatory coupling between bending into the major groove and widening of the minor groove at the site of bending. Conversely, one of the energetically effective ways to widen the minor groove at the site of insertion of any base interfering with the A_nT_m tracts is to bend the DNA in this region into the major groove. The bending is not necessarily purely roll-like and may involve some tilt. This relationship between the minor groove parameters (particularly width) and the extent of bending seems essential because these groove parameters are important for interactions with water and other molecules that may change the minor groove width by their insertion and thus alter the extent of bending.

In earlier calculations (Chuprina, 1987) the main factors responsible for increasing the width of the A_n tract minor groove from ~9 to ~12 Å at higher temperature (where the structure is more B-like) were the increased stacking between bases and the reduced electrostatic repulsion between charged phosphates (see also legend to Table IV), whereas the Dickerson spine of hydration is the cause of the narrowing of the groove to ~9 Å in A_n tracts and the stabilization of the B' conformation. The net effect of these opposing interactions results in the B' conformation in an A/T tract (without a TpA

step), for example, A₂T₂, A₃T₃, etc., and the B structure in other sequences. It is possible that there are other additional factors stabilizing the wide minor groove B conformation that are not taken into account in energy calculations, e.g., DNA interaction with the environment. Nevertheless, the formation of such a local B-like structure in the interior of B' A_nT_m tracts leads to bending into the major groove.

Influences of the Transition from B' to B Conformation on Bending. Our calculations show that there are no bends in base pair triplets when the outer two base pairs are fixed in an ordinary conformation (as opposed to an anomalous one) with the minor groove widened to ~12 Å and the τ angle unwound to ~33°. The absence of bending in such structures is largely due to the lack of both significant propeller twist and inclination in such base pairs, which results in good stacking for the trimer duplexes in conformation IV.

These results, as well as those reported in our previous studies (Chuprina & Abagyan, 1988a), explain the disappearance of the anomalous DNA electrophoretic mobility when the temperature is raised (Marini et al., 1982; Koo et al., 1986; Diekmann, 1987; Olson et al., 1988). In principle, this observation could be associated with a change in the phasing of the DNA multimer as a function of temperature. However, if the disappearance of anomalous mobility in 10 bp multimers upon heating were due simply to a loss of phasing, it should be possible to find a DNA with a different block repeat size such that the bending of each block would be *more in phase* at the higher temperature. Since the DNA repeat is always greater than 10.0 regardless of sequence, and increased temperature invariably causes underwinding, then multimers of 10 bp will always undergo a reduction in phasing as the temperature is raised and the repeat increases toward 11. However, multimers of 11 bp with a 10.1–10.5 repeat at low temperature should produce an increase of phasing with underwinding as the temperature is raised. In such cases the gel retardation should increase in parallel, but this is not the case. For (A₅N₆)_n the electrophoretic retardation is *reduced* as the temperature is raised from 4 to 20 °C (Koo et al., 1986), suggesting that the change in phasing is not the reason for the reduction in mobility. This observation suggests that reduction of the bending in the constituent blocks, rather than dephasing, is the primary cause of the disappearance of the retardation at higher temperatures.

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Registry No. d(ACA)-d(TGT), 130611-22-2; d(AGA)-d(TCT), 130573-96-5; d(ATA)-d(TAT), 90744-91-5; d(TCA)-d(TGA), 130611-23-3; d(ACT)-d(AGT), 130573-97-6.

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