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Sequence Elements Responsible for DNA Curvature

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Intrinsic DNA bending or curvature is a phenomenon that has been shown to play an important role in a variety of DNA transactions. Large curvature occurs when short homopolymeric $(dA{\cdot}dT)_{4\ 6}$ runs (A-tracts) are repeated in phase with the helical screw. We have used electrophoretic mobility modulation to examine how bending depends on the nature of the 5 bp DNA sequence between the A tracts in molecules of the form $(A_{5\ 6}N_5)_n$. We show that A-tract-induced DNA curvature can indeed be affected by other sequence elements, although by only about $\pm 10\%$. The small observed curvature modulation implies that the overall helix axis deflection contributed by 5-bp *B*-DNA segments between A-tracts varies little from one sequence to another. This result validates, to first order, the assumption that DNA curvature results from inserting A-tracts at integral turn phasing into generic *B*-DNA. Therefore, if, as has been proposed, A-tracts have zero roll between the base-pairs and all curvature results from positive roll in the *B*-DNA segments, then this must be a general property of ~ 5 bp *B*-DNA sequences, not just special cases. This interpretation would require that the canonical structure of *B*-DNA be revised to include systematic roll between the base-pairs of about 6°. Alternatively, the data are also consistent with zero average roll in the *B*-DNA sequences, and wedge angles dominated by negative roll in the A-tracts, or with an appropriate mixture of the two models. It is not possible to resolve this ambiguity using comparative electrophoresis or existing structural data. We show that published wedge angle parameters successfully predict the measured direction and, with appropriate rescaling, the magnitude of curvature due to a non-A-tract sequence containing the protein-free *lac* operator CAP protein binding site.

Keywords: bending; junction; wedge; cyclization; A-tract

It is now well known (reviewed by Crothers *et al.*, 1990) that short (4 to 6 bp) runs of adenines ("A tracts"), repeated with the helical screw (~ 10.4 bp/turn), produce a large global curvature of the DNA double helix. Such curvature has been shown to affect transcription initiation *in vivo* (McAllister & Achberger, 1989; Bracco *et al.*, 1989; Pérez-Martin & Espinosa, 1993) and *in vitro* (Gartenberg & Crothers, 1991). Current models advanced to explain intrinsic DNA bending on the molecular level are usually grouped into one of two categories: (1) junction models (Selsing *et al.*, 1979; Wu & Crothers, 1984; Levene & Crothers, 1983), in which two distinctly different helical segments having different base-pair angular orientations relative to their respective overall (straight) helix axes meet at a "junction". Preservation of base stacking at the junction causes the two helix axes to be non-parallel; no base-pair roll or tilt is required at the junction itself. Cooperative buildup of a distinctive helical structure has been demonstrated for dA·dT tracts (Leroy *et al.*, 1988; Haran & Crothers, 1989; Nadeau & Crothers, 1989). In the statement of the junction model by Koo *et al.* (1986), it was proposed on the basis of early fiber diffraction studies of poly(dA)·poly(dT) that the base-pairs in A-tracts have negative inclination relative to the overall helix axis, resulting from negative roll between the base-pairs. This model, with small revisions to account for the lack of full dyad symmetry of A-tracts (Koo & Crothers, 1988; reviewed by Crothers & Drak, 1992), remains consistent with the observed direction of bending (Crothers *et al.*, 1990; Crothers & Drak, 1992) and with revised models for the structure of poly(dA)·poly(dT) (Lipanov *et al.*, 1990). Alternatively, it has been proposed that the bends result from interspersing A-tracts having zero roll with *B*-DNA segments having positive roll between the base-pairs (Calladine *et al.*, 1988; Maroun & Olson, 1988; Goodsell *et al.*, 1993, 1994; Grzeskowiak *et al.*, 1993; Dickerson *et al.*, 1994), a proposal that is also consistent with the experimental direction of bending in solution. Comparative electrophoresis measurements cannot distinguish such models.

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Category (2) consists of wedge models (Trifonov, 1980; Trifonov & Sussman, 1980; Bolshoy *et al.*, 1991; De Santis *et al.*, 1992), which propose smooth global bending as a result of small additive wedges, composed of the roll and tilt components of independent base-pair steps. All 16 wedge angles have been estimated from extensive experimental measurements of DNA gel mobility (Bolshoy *et al.*, 1991; De Santis *et al.*, 1992). Several dinucleotide steps other than AA are found to deflect the DNA axis appreciably.

The distinction between junction and wedge models has frequently been misunderstood. Both models can be used to describe the same structure. The primary difference is in how the helix axis is defined. The junction model defines separate overall, straight helix axes for A-tracts and adjacent *B*-DNA segments, even though because of base-pair roll there may be writhe of the local helix axis around the overall axis. At the junction, the overall A-tract helix axis is deflected because after the junction it describes a different helical segment, namely that of the adjacent *B*-DNA. This does not, however, require that there be any wedge angle at all between the base-pairs at the junction, as is sometimes mistakenly assumed. In the wedge model the bend is smoothly distributed over the contributions from the local, writhing helix axis. In the junction model these cumulative bending effects are accounted for by the helix axis deflection at the junction. That model further holds that there may be *B*-DNA sequences that can modulate the extent of bending of the double helix, but that such effects are small compared with the special effect produced by interspersing generic *B*-DNA with dA·dT tracts, and can be ignored to first approximation (Koo *et al.*, 1986). In wedge models the AA wedge is not unique, but is only one of a number of dinucleotide steps capable of causing DNA to bend. Far too much has been made of the essentially trivial differences between these two complementary ways of describing DNA curvature.

The first objective of this paper is to assess the extent to which *B*-DNA sequences can modify A-tract-induced bending; the second is to test current solutions of wedge angles for the 16 dinucleotide steps (Bolshoy *et al.*, 1991; De Santis *et al.*, 1992) and their power to predict the magnitude and direction of curvature.

The sequences of intervening B*-DNA segments have modest effects on the curvature of A-tract containing DNA*

The sequences examined (Table 1), characterized initially according to the wedge angle parameters of Bolshoy *et al.* (1991), include three types of fragments: Ast, representing a "standard A-tract motif" used in previous studies (Koo & Crothers, 1988; Koo *et al.*, 1990), Agood, predicted to enhance A-tract bending, and Abad, predicted to diminish A-tract bending. Since there is no routine way to use the wedge parameters to design sequences with a desired curvature, the sequences are not necessarily those that will cause maximal possible curvature modulation. After the first set of experiments, the sequences Ag2R and Ab2R were designed to test the models further. We are grateful to E. Trifonov and A. Bolshoy for suggesting the Abad, Agood, Ag2R, and Ab2R sequences.

When comparing apparent DNA curvature by electrophoresis, it is important to account for possible changes in helical repeat as well as changes in curvature. Therefore the sequences (except Ag2R and Ab2R) were synthesized with four different sequence repeats: 30, 31, 21, and 32 bp (3 × 10.0, 3 × 10.33, 2 × 10.5, and 3 × 10.67, respectively). The multimers should be most retarded when the sequence repeat is equal to the helical repeat of that sequence (Drak & Crothers, 1991). By observing the electrophoretic anomaly at its maximum, we can deconvolute the effects on mobility arising from a change in curvature as opposed to an altered helical repeat. In ascribing the electrophoretic retardation to curvature, we implicitly assume that the molecules compared do not differ enough in flexibility to require consideration of that variable, since excess flexibility has been shown to contribute, albeit modestly, to the electrophoretic anomaly (Kahn *et al.*, 1994). While it is not possible to prove this assumption rigorously, the available data indicate that A-tract containing molecules do not differ appreciably in persistence length from normal DNA (Koo *et al.*, 1990), for which no dependence of flexibility on sequence has been established.

Figure 1 shows R_L values (apparent chain length/real chain length) *versus* chain length for Agood, Abad, and Ast, and Amix (see below) at each sequence repeat, and Figure 2 shows the value of R_L at a fixed chain length (147 bp) as a function of sequence repeat. At the helical twist corresponding to maximal anomaly in Figure 2, the values of $R_L - 1$ are

Table 1

Sequences used in this work

Name	Sequence
Agood21	$GACTA_6TGACTA_5T$
Abad21	$TCGGA_6GTCGGA_5G$
Amix21	$TCGGA_6TGACTA_5G$
Ast21	$GGGCA_6CGGGCA_5C$
Agood30	$GACTA_5TGACTA_5TGACTA_5T$
Abad30	$TCGGA_5GTCGGA_5GTCGGA_5T$
Ast30	$GGGCA_5CGGGCA_5CGGGCA_5C$
Agood31	$GACTA_5TGACTA_5TGACTA_6T$
Abad31	$TCGGA_5GTCGGA_5GTCGGA_6G$
Ast31	$GGGCA_5CGGGCA_5CGGGCA_6C$
Agood32	$GACTA_5TGACTA_6TGACTA_6T$
Abad32	$TCGGA_5GTCGGA_6GTCGGA_6G$
Ast32	$GGGCA_5CGGGCA_6CGGGCA_6C$
Ag2R31	$TGAGA_5CTGAGA_5CTGAGA_6C$
Ab2R31	$GTGTA_5GGTGTA_5GGTGTA_6G$
Ag2R32	$TGAGA_5CTGAGA_6CTGAGA_6C$
Ag2R21	$TGAGA_6CTGAGA_5C$

Only one strand in each duplex is shown. All duplexes were constructed with 2 base-pair 5'-protruding sticky ends.

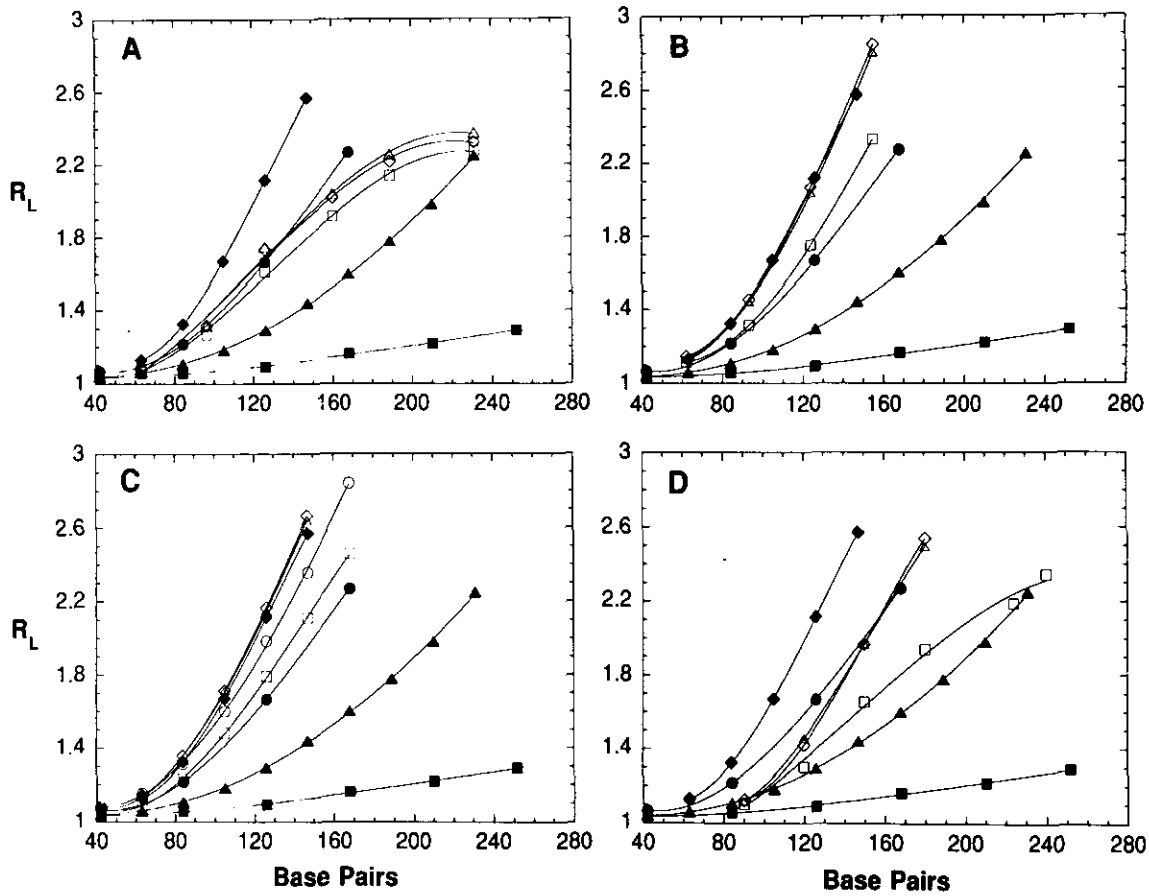

Figure 1. R_L values *versus* actual chain length for: A, fragments with sequence repeat 30; B, 31; C, 21; and D, 32 bp. The open symbols represent the data examined here: diamonds, Agood; squares, Abad; triangles, Ast; and circles, Amix. The filled symbols represent the data from fragments incorporating different numbers of A6 tracts per helical turn, included here as bend magnitude markers: $A_6$1/1 (diamonds) represents 1 A_6 tract/helical turn, which is equivalent to an 18° bend (Koo *et al.*, 1990), $A_6$3/4 (circles) represent 3/4 A_6 tract/helical turn, $A_6$1/2 (triangles) represent 1/2 A_6 tract/helical turn, and $A_6$1/4 (squares) represent 1/4 A_6 tract/helical turn. Deoxyoligonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems). Oligonucleotides were deprotected and purified as previously described (Haran & Crothers, 1989). A total of 4 μg of each single-stranded DNA oligomer was 5'-labeled with 25 μCi of [γ-^{32}P]ATP (New England Nuclear, spec. act. > 3000 Ci/mmol) and 2.5 units of T4 polynucleotide kinase (New England Biolabs) in a 10 μl reaction for 30 min at 37°C, followed by 1 mM ATP and 2.5 more units of T4 polynucleotide kinase for an additional 30 min at 37°C. This was followed by purification as previously described (Haran & Crothers, 1989) and hybridization of complementary oligonucleotides. 60% of the hybridized oligonucleotides were multimerized with 2 units of T4 DNA ligase at 16°C overnight, followed by the addition of the remaining 40% of the hybridized oligonucleotides and 1 more unit of ligase and further incubation at 16°C for 2 h. The ligation products were analyzed on a native 8% (w/v) polyacrylamide gel (acrylamide:bisacrylamide 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 24 cm in a 20 cm × 38 cm × 0.8 mm gel.

1.7 for Agood and 1.18 for Abad. Given that curvature depends quadratically on $R_L - 1$, we deduce that the curvature of Abad is 83% as large as that of Agood (Koo & Crothers, 1988; Calladine *et al.*, 1988; Koo *et al.*, 1986). Hence there is a variation of about ±10% around the average curvature due to changing the *B*-DNA segments.

In order to avoid overlooking a significant change in bend direction due to exchange of *B*-DNA segments, we constructed a fourth type of DNA fragment, called Amix (Table 1), where the Agood and Abad *B*-DNA regions alternate between the A-tract regions. The multimers of this sequence have gel-anomaly properties that are between those of Agood and Abad (Figure 1C, Table 2). Had the overall direction of bending been changed between Agood and Abad, their curvatures would have tended to cancel each other and the mobility of Amix would have been more "normal" than the average of the two (i.e. like that of straight DNA; Koo *et al.*, 1986).

Finally, the sequence Ab2R31, predicted by the Bolshoy *et al.* (1991) parameters to be of higher mobility (lower anomaly) than Agood or Ast, has indeed changed in the right direction, but the predicted change is larger than the observed change in gel mobility or curvature. The curvature of Ag2R31 changed in the opposite direction from that predicted; we verified by synthesis of two more fragments, Ag2R21 and Ag2R32 (Table 1), that this was not because of a change in helical twist.

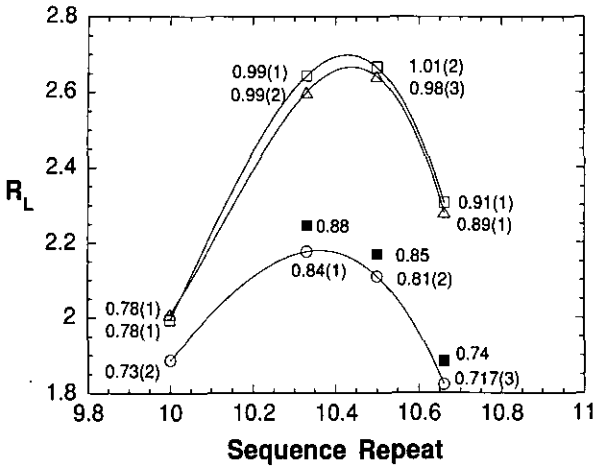

Figure 2. R_L values *versus* sequence repeat for multimers of chain length 147 base-pairs. The maximum of each graph represents the apparent helical twist of that sequence. The numbers near each graph correspond to DNA curvature relative to $A_6N_4A_6N_5$ (Koo *et al.*, 1990), averaged over 5 experiments. The open squares, Agood; open circles, Abad; open triangles, Ast; and the filled squares are for Ag2R.

Modulation of curvature by sequence changes outside the A-tracts has also been suggested by the results of selection-amplification studies (Beutel & Gold, 1992). In particular, the dinucleotide CA/TG was found to affect apparent curvature, as has also been proposed by Nagaich *et al.* (1994). Koo *et al.* (1986) reported that changing the nucleotides flanking the A-tract from C and T to T and T or G and G had minor but detectable modulating effects on curvature. It is worth noting in addition that all of the sequences found to be curved in the selection-amplification experiments contained phased A-tracts, with a considerable variability of the sequence between them. This suggests that, with the possible exception of the CA step, there may not be other sequences that have an important role in bending DNA. The results further imply that the primary source of curvature is the phased alternation of A-tracts and generic *B*-DNA, as our experiments also indicate.

Solutions for DNA wedge angles explain general trends in the experimental data

In Figure 3 and Table 2, we compare the experimental results with the theoretical predictions of the wedge models of Bolshoy *et al.* (1991) and of De Santis *et al.* (1992). The Figures show how the theoretical curvature varies with the values measured experimentally. The experimental values are expressed in terms of absolute curvature, following the convention of Bolshoy *et al.* (1991) that the curvature of DNA in the nucleosome is 1.0, which is equivalent to an 80 bp circle. Relative curvatures were calculated using the assumption that the electrophoretic anomaly $R_L - 1$ varies with the square of the curvature (Koo & Crothers, 1988). Conversion from relative to absolute curvature uses the calibration of the junction model parameters established by the cyclization kinetic experiments and computer simulations described by Koo *et al.* (1990), using the sequence $(A_6CGGGCA_6CGGC)_n$. Both the Bolshoy and De Santis parameters overestimate curvature according to this conversion. We therefore applied a scaling factor to the wedge angles in the parameter sets in order to bring the average theoretical curvature into agreement with the experimental value; the factor was 0.63 for the Bolshoy parameter set, and 0.72 for the De Santis parameters.

The Bolshoy parameters generally predict the correct sign for the curvature change induced by different *B*-DNA sequences, but they overestimate

Table 2

Experimental results versus *wedge model predictions*

Sequence	Exptl R_L	Pred.[a] R_L	Exptl curv. (cu)	Pred.[a] curv. (cu)	Pred.[b] curv. (cu)	Exptl change in curv. (%)	Pred.[a] change in curv. (%)	Pred.[b] change in curv. (%)
Agood21	1.42	1.62	0.39	0.72	0.61	9	31	12
Agood31	1.42	1.65	0.39	0.72	0.53	9	31	-2
Ast21	1.39	1.39	0.37	0.63	0.52	6	13	-4
Ast31	1.40	1.39	0.38	0.59	0.50	7	7	-8
Abad21	1.28	1.14	0.32	0.37	0.58	-9	-34	7
Abad31	1.29	1.13	0.32	0.31	0.49	-7	-44	-11
Amix21	1.34	1.34	0.35	0.49	0.60	0	-12	10
Ag2R31	1.30	1.66	0.33	0.63	0.56	-6	+15	3
Ab2R31	1.27	1.15	0.31	0.51	0.51	-10	-7	-6

Experimental R_L (interpolated) values correspond to 90 bp long fragments of DNA. Predicted curvature values are expressed in curvature units, cu (Bolshoy *et al.*, 1991). A superscript *a* indicates that the Bolshoy *et al.* (1991) parameter set was used for a prediction, *b* indicates the De Santis *et al.* (1992) parameter set. Predicted R_L values are calculated from the calibration curve for anomaly *versus* curvature of Bolshoy *et al.* (1991). Experimental curvature values are calculated assuming that $(R_L - 1)$ depends quadratically on curvature, referenced to curvature = 0.379 cu for Ast31 as determined by cyclization kinetics (Koo *et al.*, 1990). Observed and predicted changes in curvature are related to the average value of curvature (cu) for the 9 A-tract fragments listed. Predicted curvature was calculated from wedge angles essentially as by Bolshoy *et al.* (1991), using standard lengths of 95 bp for the Bolshoy parameters (to avoid truncating A-tracts) and 145 bp for the De Santis parameters (to avoid errors otherwise caused by small changes in writhe).

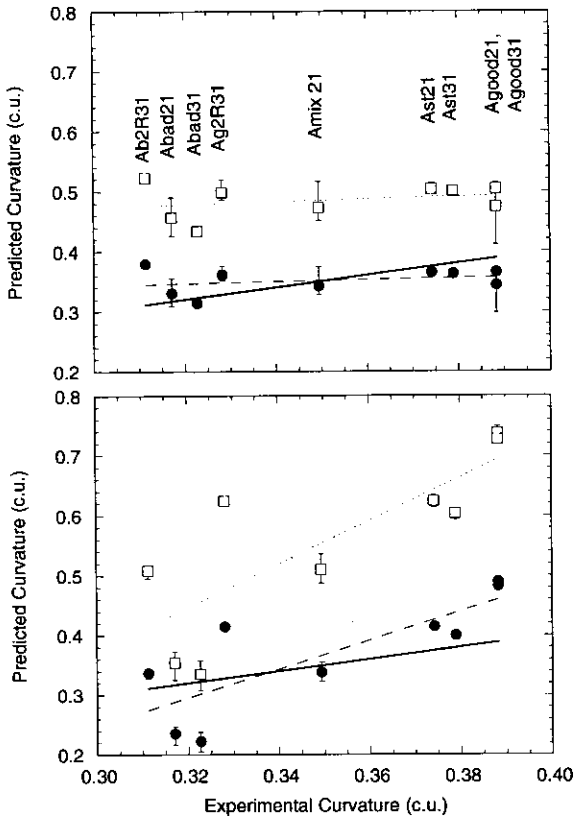

Figure 3. Comparison of observed and predicted DNA curvature, using the wedge angle parameters of Bolshoy *et al.* (1991) (lower panel) and De Santis *et al.* (1992) (upper panel). The experimental curvature values of Table 2 constitute the x axis. These same values are plotted as the continuous line for comparison, showing the ideal result of an exact theoretical prediction. The theoretical curvature values (before rescaling) are shown by hollow squares. The range of values for different assumed helical repeats (see below) is shown by the length of the "error" bar associated with each point. The dotted lines shows the best-fit correlation of the predictions with the experimental data (correlation coefficient $R = 0.80$ for the Bolshoy parameters; $R = 0.25$ for the De Santis predictions). Theoretical curvatures rescaled to bring the mean value into agreement with experimental results are shown as filled circles and broken lines. The "scaling factors", i.e. the average ratios between the curvature predicted by the wedge angle parameters and by the cyclization experiments, are 0.63 and 0.72 for the Bolshoy and De Santis parameters sets, respectively. For Ast31, which is most similar to the molecules studied by cyclization, the calibration factors are 0.64 and 0.76, respectively. The junction model (Koo *et al.*, 1990), which ignores the sequence dependence of the *B*-DNA segments, shows only minor variations in predicted curvature (not shown), resulting from the changes in sequence repeat. Theoretical curvature values using the Bolshoy and De Santis wedge angles were calculated essentially as by Bolshoy *et al.* (1991). We assumed the DNA helical repeat to be 10.33 or 10.45 or used the twist angles specified by the wedge models (De Santis *et al.*, 1992; Kabsch *et al.*, 1982). Sequences with a sequence repeat of 21 bp show more variability due to the increased contribution from writhe, which is increased when the sequence repeat does not match the helical repeat.

the total curvature and the extent of curvature modulation by non-A-tract DNA. The De Santis parameter set predicted an average curvature closer to the value from cyclization kinetics, and the overall variation in the curvature of the different sequences is smaller than the variation obtained with the Bolshoy parameters, closer to that of the experimental data, but the signs of the changes induced by different *B*-DNA segments are not predicted accurately. The correlation coefficient for theoretical prediction and experimental results is 0.25 for the De Santis *et al.* parameters, and 0.8 for the Bolshoy *et al.* parameters, calculated before application for the scaling factor.

To avert misunderstanding we stress that the wedge angles discussed here measure deflections of the DNA helix axis at each dinucleotide step. They should not be confused with structural data obtained at atomic resolution showing actual roll or tilt angles between base-pairs. The DNA base-pairs may have systematic average tip and inclination angles (relative to the overall helix axis). When the axis deflection is calculated, as a difference between two steps, these constant angles do not matter. In the junction model what matters is also the difference in base-pair inclination and tip between A-tracts and the interspersed *B*-DNA segments.

Bending results from a difference in structure between A-tracts and B*-DNA*

We conclude that in A-tract molecules most of the intrinsic DNA curvature observed is due to the presence of phased A-tract regions, with minor modulations in both amplitude ($\pm 10\%$) and direction by changing the character of other sequence elements. This result validates, to first order, the assumption of the junction model that bending results from a difference in structure between A-tracts and generic *B*-DNA.

If, as has been suggested (Calladine *et al.*, 1988; Maroun & Olson, 1988; Goodsell *et al.*, 1993, 1994; Grzeskowiak *et al.*, 1993; Dickerson *et al.*, 1994), DNA curvature results from straight A-tracts with zero roll, and positive roll in the *B*-DNA segments, then our results have the following consequence: all ~5 bp *B*-DNA segments examined, regardless of sequence, are required to have an average base-pair inclination of $9(\pm 1)°$ relative to their overall helix axes, in order to create the measured bend angle of $18°(\pm 10\%)$ resulting from the difference in base-pair inclination at each of two junctions per A-tract (Koo *et al.*, 1990). A roll angle between the base-pairs in the *B*-DNA segments of about 6° is needed in the wedge model to generate this base-pair inclination. Since there is no evidence indicating that the DNA bend per A-tract is reduced by increasing the length of the adjacent *B*-DNA segment from 0.5 to 1.5 helical turns (Koo & Crothers, 1988), it is likely that this requirement extends also to *B*-DNA segments longer than 5 bp. This interpretation therefore requires a significant revision in the structure of generic or canonical *B*-DNA.

While we continue to regard this model as a formal possibility (Crothers *et al.*, 1990), the available structural data for a variety of sequences do not support such an hypothesis, notwithstanding the observation of a bend in a specific (non-A-tract) sequence in a crystal (Goodsell *et al.*, 1993). It still seems to us more likely that the *B*-DNA base-pair inclination averages close to zero as in the canonical structure, while the base-pair inclination in the A-tracts is close to $-9°$; systematic roll of about $-6°$ is required in the language of the wedge model to generate this inclination. However, it is only the difference in base-pair inclination (or roll) that one can be sure of: it must average close to 9° (or 6° of roll), regardless of *B*-DNA sequence, and any combination of the two models that yields the appropriate inclination (or roll) difference is in principle acceptable at the present stage of knowledge. A compromise example would be 3° of positive roll in *B*-DNA, and 3° of negative roll in the A-tracts.

Dickerson and colleagues (Goodsell *et al.*, 1993, 1994; Grzeskowiak *et al.*, 1993; Dickerson *et al.*, 1994) have recently reached a conclusion differing from ours, which can be summarized by their statement that the model in which A-tracts have zero roll and the *B*-DNA segments have positive roll "... must be regarded as the only consistent model for A-tract bending" (Goodsell *et al.*, 1994). This conclusion is based partly on X-ray structures, and partly on their analysis of comparative electrophoresis experiments. They argue that because the only structure for an A-tract that has ever been seen in a crystal has nearly zero roll between the base-pairs, that structure must be considered to be the basis for bending. However, thermochemical, optical and NMR experiments (Herrera & Chaires, 1989; Chan *et al.*, 1990, 1993; Park & Breslauer, 1991; Nadeau & Crothers, 1989; Nadeau & Crothers, unpublished) show that the structures of both poly(dA)·poly(dT) and A-tracts are temperature-dependent. Indeed, DNA bending by A-tracts is greatly reduced at higher temperatures (Koo *et al.*, 1986; Diekmann, 1987), implying the existence of a structural form that does not cause bending.

In the face of such clear evidence that A-tracts are polymorphic, in solution if not in the crystal, we are not persuaded that the one structure that has been seen must necessarily be that responsible for bending. The history of the field shows that it is dangerous to assume that the DNA structure found in a crystal must be the predominant form in the supernatant. It should also be emphasized that none of the crystal structures solved for A-tract-containing molecules (Nelson *et al.*, 1987; Coll *et al.*, 1987; DiGabriele *et al.*, 1989; DiGabriele & Steitz, 1993) has an overall bend direction that conforms to the results found by comparative electrophoresis (reviewed by Crothers & Drak, 1992). (This corrects the unsubstantiated and unfortunately phrased assertion by Dickerson *et al.* (1994) that it is only the assumptions of the junction model, not the comparative electrophoresis experimental results, which disagree with the direction of bending in the crystal.) Additionally, proton–proton distances within the A-tract measured by NMR (Nadeau & Crothers, 1989) agree better with the model derived from fiber diffraction (Alexeev *et al.*, 1987), in which there is negative roll between the base-pairs, than with the dimensions in the crystal studied by DiGabriele & Steitz (1989). The crystallographic work of Coll *et al.* (1987) revealed a typical propeller twisted A-tract structure in the sequence $CGCA_3T_3GCG$ complexed with distamycin, which is a drug that has been shown to remove DNA curvature (Wu & Crothers, 1984). This result invites consideration of the possibility that the A-tract structure seen in crystals may actually be the form corresponding to unbent DNA.

Finally, the crystallographic evidence does not broadly support the view that there is a systematic roll angle of about 6° between the base-pairs in generic *B*-DNA, as is required by our results when taken together with he model that bending results from roll in the *B*-DNA segments. From these considerations we conclude that the "roll in *B*-DNA only" model is based on a selective rather than unbiased interpretation of the structural results, and should not be preferred over alternative models at this time.

Goodsell *et al.* (1994) also make an effort to use comparative electrophoresis results to eliminate models in which the predominant wedge angles are in the A-tracts. They set up a straw-man model in which bending must be exclusively by roll, and then demolish it by showing that the resulting dyad symmetry of A-tracts is not consistent with the properties of sequences such as those studied by Hagerman (1986, 1988). However, it has been recognized for many years on the basis of those and other sequences, along with NMR data, that the structure of A-tracts cannot be fully dyad symmetric (Koo *et al.*, 1986; Ulanovsky & Trifonov, 1987; Koo & Crothers, 1988; Nadeau & Crothers, 1989; Crothers & Drak, 1992). Nor is there any sound energetic or structural basis for excluding a modest tilt contribution at A-A steps. The corresponding adjustments have been made in the parameters both for the wedge model (Ulanovsky & Trifonov, 1987), where a small tilt component must be added to the roll wedge, and for the junction model (Koo & Crothers, 1988), for which a helix axis deflection in the direction of roll must be added at the junction. Both of these models account adequately for the electrophoretic anomalies of the previously described A-tract-containing molecules. Apparently the basic principle needs repeating: comparative electrophoresis cannot be used to distinguish models that differ in the underlying structural basis for bending, unless the direction of bending is incorrect, as is the case, for example, in known crystals containing A-tracts. Ultimate resolution of the structural problem will probably require accurate determination of A-tract structure(s) in solution, or a crystal structure that explains the solution polymorphism of A-tracts and poly(dA)·poly(dT), or a crystal structure of an A-tract-containing molecule in which the overall bend direction conforms to the results in solution, or all of these. Adherents of the model with zero roll in the A-tracts will need to find crystallographic

support for revising the structure of generic *B*-DNA to include the necessary roll angles.

Adequacy of wedge angle models

The inability of wedge models to predict quantitatively the changes in curvature that we observe may reflect an insufficiency of sequences so far. (Although the number of sequences used for the Bolshoy *et al.* (1991) parameters now exceeds the number of free parameters by nearly fourfold, the dinucleotide composition of bent sequences is heavily weighted toward AA.) Alternatively, and perhaps more likely, it may reflect an underlying dependence of wedge angles on structural elements larger than dinucleotides. In support of this view, recent experiments on DNA bending in AP-1 and CREB recognition sites revealed DNA curvature that is not predicted by the dinucleotide models (Paolella *et al.*, 1994). Further, the sequence GGGCCC has been shown to bend toward the major groove at its center, to an extent also not predicted by the dinucleotide parameters (Brukner *et al.*, 1993). Curvature is particulary pronounced in the presence of divalent ions (Brukner *et al.*, 1994). A trinucleotide or higher model would be called for if, for example, wedge angles at dinucleotides in or between A-tracts are affected by their context (Carrera *et al.*, 1991). Many more sequences than now available would be needed to solve a trinucleotide model. Repetitive sequence elements, as studied in the present experiments, would exacerbate this difficulty, since the dinucleotide context is repetitive rather than variable.

Cyclization kinetics can be used to test the predictions of models for DNA bending

We therefore asked, how accurate is the parameter set in predicting the curvature of a non-repetitive DNA sequence, in which the properties of dinucleotides are better averaged over context than in ligated multimers? Cyclization kinetic assays of DNA molecules containing A-tracts coupled with the *Escherichia coli* CAP (catabolite activator protein) binding site provide data on the curvature of a "typical" DNA sequence, relative, in both direction and magnitude, to A-tract bends. Experiment (Kahn & Crothers, 1992) shows that a 156 bp DNA molecule (denoted 09T15) with the CAP site "in phase" with the A-tracts cyclizes 13 times more efficiently, in the absence of the protein, than does the 156 bp molecule 15A09, in which the CAP site is out of phase. In order to simulate the results quantitatively (essentially as described by Koo *et al.* (1990); Kahn & Crothers, unpublished), the intrinsic curvature of the CAP site was modeled with a pair of 7.5° roll bends one base-pair upstream of the positions at which the kinks are observed in the crystallographic structure of the complex (Schultz *et al.*, 1991). This, along with the junction model for A-tracts used in earlier simulations (Koo *et al.*, 1990), yields a ratio of cyclization efficiencies (*J* factors) of 12. The Bolshoy *et al.* (1991) wedge parameters (with all wedge angles scaled by a factor of 0.61 to give agreement with the experimental curvature for the specific A-tract sequence contained in the cyclized fragment), yielded a ratio of 10, and fit the entire free-DNA data set of Kahn & Crothers (1992) equally as well as did the empirically-derived roll-bend model above. Thus, we find that the (rescaled) parameter set accurately predicts both the magnitude and direction of curvature of a non-repetitive DNA sequence. The De Santis *et al.* (1992) parameter set, scaled as for the Bolshoy *et al.* set to give the correct extent of A-tract bending, predicted the same direction of curvature for the CAP site, but about twice the magnitude.

In conclusion, our results support the junction model's assumption that all A_{5-6} tracts produce the same curvature, regardless of context, to an accuracy of about $\pm 10\%$. Unfortunately, there is no way at present to determine the extent of error in the wedge model in assuming that all AA wedges are the same, whether or not they are in longer A-tracts. Undoubtedly, non-A-tract sequences can produce curvature, and indeed the estimated wedge angles for AG, CG and GC are comparable to the AA-wedge. One obvious advantage of the AA (TT) dinucleotide step in the design of curved DNA molecules, even though its wedge angle may not be the largest, is the unique possibility of tight clustering of the AA wedges in A-runs. None of the other dinucleotide elements can act this way, since the CC (GG) wedge is near zero and the others are hetero-dinucleotides for which the next step usually tends to have a compensatory wedge angle. In this sense the wedge and junction models are convergent.

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