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Analysis of Local Helix Bending in Crystal Structures of DNA Oligonucleotides and DNA-Protein Complexes

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ABSTRACT Sequence-dependent bending of the helical axes in 112 oligonucleotide duplex crystal structures resident in the Nucleic Acid Database have been analyzed and compared with the use of bending dials, a computer graphics tool. Our analysis includes structures of both A and B forms of DNA and considers both uncomplexed forms of the double helix as well as those bound to drugs and proteins. The patterns in bending preferences in the crystal structures are analyzed by base pair steps, and emerging trends are noted. Analysis of the 66 B-form structures in the Nucleic Acid Database indicates that uniform trends within all pyrimidine-purine and purine-pyrimidine steps are not necessarily observed but are found particularly at CG and GC steps of dodecamers. The results support the idea that AA steps are relatively straight and that larger roll bends occur at or near the junctions of these A-tracts with their flanking sequences. The data on 16 available crystal structures of protein-DNA complexes indicate that the majority of the DNA bends induced via protein binding are sharp localized kinks. The analysis of the 30 available A-form DNA structures indicates that these structures are also bent and show a definitive preference for bending into the deep major groove over the shallow minor groove.

INTRODUCTION

The crystal structure of the d(CGCGAATTCGCG) duplex was first reported in 1981 by Drew and Dickerson, the first example of the structure of a B-form DNA oligonucleotide at high resolution. Analysis of the results led to the observation of sequence-dependent axis bending in the DNA helix (Dickerson, 1983). Subsequently, studies from diverse laboratories have been directed toward extracting patterns in sequence-dependent bending and related helicoidal fine structure from the crystal structures of the d(CGCGAATTCGCG) duplex under various conditions of temperature and complexation (Bhattacharyya and Bansal, 1990; Bansal et al., 1991; Dickerson, 1992; Sundaralingam and Sekarudu, 1988). In parallel studies, the observation that runs of A's and T's in a DNA sequence occurring in phase with helix pitch were responsible for sequence-directed curvature was introduced (Marini et al., 1982). The results of these studies have wide implications in a variety of problems in molecular biology and biophysics, including the specificity of the interaction of drugs with DNA (Kennard, 1993), the nature of protein-DNA recognition complexes in the molecular mechanisms of gene expression (Steitz, 1990), and the structure of DNA in nucleosomal packages (Saenger, 1983).

Recently, the crystallographic coordinates of all oligonucleotide structures reported to date have been collected into the Nucleic Acid Database (NDB) (Berman et al., 1992), a relational data base upon which complex, computer-automated inquiries may be readily formulated and processed. This entity contains a number of derived quantities for each sequence, such as conformational and helicoidal parameters, which facilitate further systematic and longitudinal analyses of axis bending in the various structures. We describe herein calculations of local helix bending in all B- and A-form DNA oligonucleotide crystal structures reported to date, including those complexed to drugs and proteins. In particular, we have investigated the range of axis deformations at the base step level, using a graphical tool called bending dials. The results are used to present a systematic empirical view of axis bending in oligonucleotide crystal structures and to determine the extent to which the crystal structure data supports the various theories of local axis bending in the double helix. This study can also serve as a basis for a subsequent comparisons and critiques of oligonucleotide structures obtained from nuclear magnetic resonance (NMR) spectroscopy, molecular dynamics (MD) simulations, or other structure determination methods or predictions.

BACKGROUND

DNA bending has been the topic of several recent reviews (Hagerman, 1992; Sundaralingam and Sekarudu, 1988; Trifonov, 1991; Zinkel and Crothers, 1991), and we provide here only a concise overview of aspects of this subject particularly relevant to the present work. Some years ago, Zhurkin and co-workers (Ulyanov and Zhurkin, 1984; Zhurkin, 1983; Zhurkin, 1985; Zhurkin et al., 1979, 1982) conducted a theoretical analysis of sequence effects on DNA based upon empirical energy calculations. The results supported the idea that the double helix bends more easily toward the grooves than the backbone and suggested that purine-pyrimidine steps tend to bend toward the minor groove whereas pyrimidine-purine steps bend by compressing the major groove. This theory has had high predictive utilization and

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a number of notable successes in understanding bending in nucleosomal sequences (Zhurkin, 1983) and sequences with retarded electrophoresis mobility (Koo et al., 1986). Recently, Zhurkin et al. (1991) have extended their analysis to include the effect of thermal fluctuations. They compared predictions based on static structures (energy-minimized forms) and on statistical ensembles generated from Monte Carlo simulations and found consideration of fluctuations to be important, particularly in moderately and slightly curved sequences.

The analysis of the crystal structure of d(CGCGAATTCGCG) revealed a 19° bend in the helix axis, occurring in concert with base pair roll (Dickerson and Drew, 1981). The observed irregularities in the structure were correlated with intrinsic chemical differences between the four types of nucleotide bases (Dickerson et al., 1987), a conclusion furthered, at least for this class of systems, by the base-dependent steric clash arguments set forth by Calladine (Calladine, 1982; Calladine and Drew, 1986). In this structure, purine-pyrimidine steps are systematically found with negative roll values, and pyrimidine-purine steps tend to have positive roll values (Dickerson and Drew, 1981), results consistent with Zhurkin's theory. A subsequent analysis, including more data, proposed the grouping of a subset of the 16 possible two-base-pair steps into two distinct classes, high twist profile (HTP) and low twist profile (LTP) (Yanagi et al., 1991). The former class is characterized by roll compression of the minor groove (negative roll) and includes AG, AT, CG, and CT steps. The latter group is marked by positive rolls (compressing the major groove) and includes the homopurine steps AA, GA, and GG as well as the homopyrimidine steps TC, TT, and CC. Most recently, Goodsell et al. (1994) have discussed issues of base pair roll and tilt in bending of B-DNA sequences.

Analysis of the base sequence of curved kinetoplast DNA samples (Marini et al., 1982) revealed a preponderance of runs of adenine bases (A-tracts) occurring in phase with the natural helical repeat of DNA and prompted investigations into a relationship between phased A-tracts and intrinsic bending in DNA. Ideas about the relationship between sequence and bending in DNA containing A-tracts came to be embodied in the so-called "wedge model" (Ulanovsky and Trifonov, 1987) and "junction model" (Koo and Crothers, 1988; Marini et al., 1982; Wu and Crothers, 1984). In the simplest form of the wedge model, bending was proposed to occur at AA steps within A-tracts. In a junction model, helix bends occur at the articulation of A-tracts with sequences of other composition. Other special properties of A-tracts that may be related to curvature include propeller twist (larger for AT than for GC pairs), negative base pair inclination with respect to the helical axis, and minor groove narrowing associated with an embedded spine of hydration (Chuprina, 1987; Drew and Dickerson, 1981; Subramanian and Beveridge, 1989). A-tracts have thus been the subject of a large number of subsequent studies, and a succession of variations and modifications of the initial theories have been required to account for the increasing experimental data relevant to this problem (Crothers et al., 1990; Hagerman, 1992; Price and Tullius, 1992; Ulanovsky and Trifonov, 1987).

There has been refinement over time in the definition of wedge model and junction model from that of the original papers. As a consequence, we adopt herein the convention that the terms wedge model and junction model refer to useful generic classes, within which fall various specific models. In the wedge models, bending occurs at AA steps within A-tracts whereas, in the junction models, bending occurs at or near the articulation of these A-tract sequences with the flanking sequences. In the Crothers junction model, this articulation involves a specific combination of negative tilt (TLT) coupled with a positive roll (ROL), but of course other junction models are conceivable as well.

Investigation of the role of properly phased A-tracts in the sequence-directed bending of DNA has resulted in some independent predictions about DNA bending that are relevant to the current analysis. Initial interpretations of anomalous gel migration rates of double stranded DNA containing phased A-tracts assumed that intrinsic DNA bends are positioned at the junctions between the A-tracts and random sequence DNA (Koo et al., 1986; Levene et al., 1986). Limitations in the gel migration experiment made the identification of the precise location of the bend difficult, but the overall bend appeared to be in the direction of the minor groove as defined with respect to the center of the A-tract (Koo and Crothers, 1988). Model building analysis of NMR data from A-tract sequences was found to be consistent with both a junction bending model and minor groove narrowing (Nadeau and Crothers, 1989). Footprinting experiments (Price and Tullius, 1992) suggest that a concerted structural change occurs within A-tracts.

The idea of steric clashes between nucleotide base pairs in DNA was embodied in a theoretical treatment of local helix structure and macroscopic curvature presented by Tung and Harvey (Tung and Harvey, 1986). A series of papers by Olson and co-workers (Maroun and Olson, 1988a; Maroun and Olson, 1988b; Olson et al., 1988) further elucidated the problem with a theoretical treatment of sequence effects on configurational statistics of DNA polymers via matrix generator techniques. Theoretical prediction of persistence and bending properties of long but finite sequences of DNAs of various sequence compositions were made based on dimer potential surfaces for the helicoidal parameters roll, tilt, and twist, assuming independence between base pair steps. The results were found to account well for qualitative trends in DNA bending and persistence compared with observed results from gel electrophoresis experiments.

Theoretical investigations by Sarai et al. (Sarai et al., 1988) into the origin of sequence-specific helical structure concluded that the intrinsic electrostatic patterns of the base pairs dominate the stacking interactions and thus bending propensities of the B-DNA double helix. Theoretical free energy estimates on each of the 16 possible two-base-pair steps (Sarai et al., 1989) supported anisotropic bending favoring either of the groove directions. Specifically, CG, CA/TG, and TA steps were found to fluctuate about significantly

positive average roll values (~5.1°-12.3°), bending toward the major groove. GC and AT steps were found to have negative average roll values, bending into the minor groove. The energetically accessible fluctuations in roll values were approximately 8° in magnitude. Bansal and co-workers (Bhattacharyya and Bansal, 1990) have examined trends in sequence-dependent fine structure and DNA polymorphism based on crystal structure data and local helix parameters. An additional study (Nagaich et al., 1994) showed the extent to which sequence-dependent structural variations seen in oligonucleotide crystal structures can account for macroscopic bending of the DNA helix in solution.

Recently, the methods of Zhurkin et al. described above and those of Olson and co-workers (1993) have been combined to study the influence of fluctuations in roll, tilt, and twist angles on DNA curvature more extensively. They observe that the ensemble average structure of a flexible DNA is not necessarily the same as the energy minimum or static form structure, indicating that the underlying potential energy hypersurface is significantly asymmetric (nonharmonic). This observation is able to reconcile some discrepant results on bending of the representative A-tract sequence d(CGCAAAAAACGC) duplex from crystallography and NMR data. The idea of a "flexible wedge" model for helix bending was proposed. Lavery and Hartmann (1994) have recently developed a theoretical approach to sequence effects based on the JUMNA algorithm, which enumerates substates with different conformations, associated with characteristic sugar puckers.

In view of the interest in the relationship between the sequence-dependent structure of base pair steps and helix bending in DNA, and the enhanced accessibility of crystal structure data on oligonucleotides as a consequence of the emergence of the NDB, we have carried out a longitudinal study of bending in crystal structures of DNA oligonucleotides. The question we address is a simple one. What are the patterns in axis bending at the dinucleotide step level that are actually found in crystal structures of oligonucleotides, and how do these patterns tally with certain specific aspects of the various theories proposed to date? Short oligonucleotides are only slightly bent, and thus this question is of interest to the overall DNA bending problem only to a very limited extent. Nevertheless, some valuable new information and critical perspectives have been obtained.

We consider herein the available data on B-form structures and A-form structures separately. A related issue is the relationship of base sequence to DNA bending induced by the formation of complexes with drugs and regulatory proteins. Drug-bound structures contribute significantly to the database of available B-form DNAs. Sixteen relevant structures of protein-DNA complexes have been released to date, and it is already clear that protein-induced axis bending is clearly an issue of interest, an extreme case being the approximately 90° bend observed in the binding of CAP protein to its various cognate DNA sequences (Schultz et al., 1991). Barber and Zhurkin (1990) have analyzed sequence-dependent effects in CAP binding to DNA sequences and identified characteristic patterns in tetranucleotide steps, specifically that TA, CG, CA/TG, and GG/CC steps are associated with bending toward the major groove, and AT, AA/TT, and GT/AC with bending toward the minor groove. Zinkel and Crothers (1990) have utilized the DNA-bending property of the CAP protein to quantitatively measure the precise nature of the bends in the CAP-DNA complex. We include here a corresponding dinucleotide step bending analysis for the currently available protein-DNA complexes.

MATERIALS AND METHODS

The Nucleic Acids Database (NDB) is a comprehensive relational data base of three-dimensional structural information on oligonucleotides, based on crystal structures as reported from diverse laboratories. A summary of the contents of the NDB used in this study is given in Table 1. Details of the NDB are described in a recent article by Berman et al. (1992). The program NDBQUERY (Westbrook et al., 1992) was used to subdivide the database in the relevant groups for analysis. The results, including the structural and citation data as well as the helicoidal analysis of each step as performed by the program CURVES, devised by Lavery and Sklenar (1988), are saved as NDB reports.

To analyze axis bending in a given structure, we follow Zhurkin (1985) and Olson and co-workers (1988) in relating the magnitude and directionality of bending to independent deviations in the helicoidal parameters roll (ρ) and tilt (τ). A simple definition of bending in terms of the angle of axis deflection θ and its orientation relative to the major groove ϕ is given in terms of ρ and τ for a base pair step as

$$\theta = \sqrt{\rho^2 + \tau^2}, \tag{1}$$

and

for $\rho > 0$:

$$\phi = \tan^{-1}\left(\frac{\tau}{\rho}\right) \text{ when } \tau > 0 \tag{2a}$$

$$\phi = 360 + \tan^{-1}\left(\frac{\tau}{\rho}\right) \text{ when } \tau < 0, \tag{2b}$$

for $\rho < 0$:

$$\phi = 180 + \tan^{-1}\left(\frac{\tau}{\rho}\right). \tag{2c}$$

These equations are accurate descriptions of bending magnitude and direction for low angles of ρ and τ. In this study, the values of ρ and τ are computed with reference to a global helical axis generated by the CURVES procedure.

In the spirit of caveat emptor, we note that a bending analysis performed on the basis of a local helix axis, as in the program NEWHELIX (Dickerson, 1993), could produce slightly altered numerical results from that obtained from a global axis reference frame as assumed herein. The issue is as follows. Consider a base pair step, located within two sequences of distinct

TABLE 1 Current contents of the NDB as of 11 May 1994

Category	Entries used
All	112
B-form DNA	66
P $2_12_12_1$	56
10-mers	16
12-mers	51
Drew sequence	25
Protein-DNA complexes	16
A-form DNA	30

curvature, in which the absolute orientation of the individual base pairs with respect to each other is fixed. In a local axis convention, helicoidal analysis would result in specific numerical values for ROL and TLT. Hence, bending parameters θ and ϕ would be the same regardless of the molecular context. In the global approach, the same specific orientation may in fact result in slightly (but not dramatically) different ROL and TLT, and thus θ and ϕ values for the step in different contexts. The issue of which is best is unalterably a judgment call. Proponents of global analysis argue that, as the curvature of the axis is in fact indisputably different, and the parameters are defined with respect to this axis, it is sensible that ROL and TLT, and the axis bending parameters θ and ϕ should reflect this. The bending is different; therefore, the bending parameters should be different. Following the arguments presented by Lavery and Sklenar (1988), we have become in our studies (Ravishanker et al., 1989) proponents of the global reference frame. However, we have repeated all analysis based on the global frame values of TLT and ROL using the local reference frame computed by the CURVES program. We found the local axis bending results to be highly correlated with the global axis bending results. The results from the local axis bending analysis are thus consistent with all trends discussed in this paper.

The conventions involved in defining these parameters and the way in which they relate to the major groove, minor groove, and backbone of the double helix are indicated in Fig. 1. The τ parameter for a given step can be measured with reference to either of the two backbone strands. Because the positive direction is defined by the choice of the reference strand, τ values identical in magnitude but opposite in sign are computed for a step that accounts for the overall symmetry in the bending dials as presented here. The ρ parameter for a given step is independent of the choice of the reference 5′-3′ strand. Note that, to honor protocols established for DNA structures (Dickerson et al., 1989), we have hereby changed the presentation conventions from previous articles (Beveridge and Ravishanker, 1994; McConnell et al., 1994; Young et al., 1994); the principal difference to the reader is that bending into the major groove is now at the north position on the dial rather than south. We have also changed notation from previous papers, from β to θ and α to ϕ, to avoid redundancies with IUPAC designations for other nucleic acid conformational parameters.

The values of ρ and τ for a given base pair step in a sequence can be conveniently displayed as a point on a polar plot in which θ is the radial coordinate and ϕ is the angular coordinate. A schematic of this plot, henceforth referred to as a bending dial, is shown in a perspective view at the lower right hand corner of Fig. 1. The bending analysis of the structure of a sequence available as a NDB report can thus be performed readily on this basis, and the results of multiple sequences can be superimposed and compared on a single set of dials. Bending dials are a prospective component of the suite of programs in Molecular Dynamics ToolChest (Ravishanker and Beveridge, 1994).

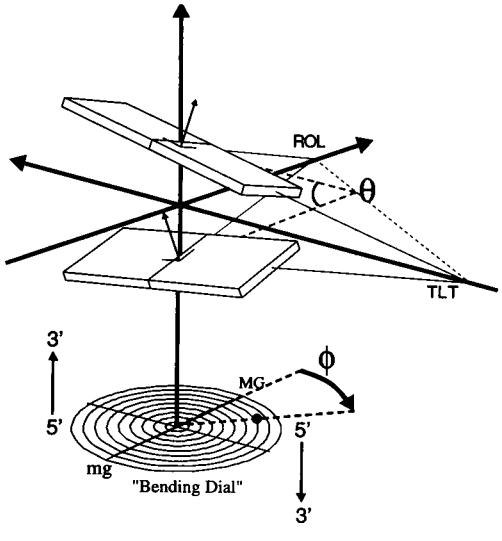

FIGURE 1 Schematic definition of the axis bending direction ϕ and bending magnitude θ in terms of the helicoidal parameters roll (ρ) and tilt (τ), defined with respect to the major groove (MG) and minor groove (mg) of the DNA double helix. Positive roll (bending compressing the major groove) is plotted in the northern hemisphere on the dial, and negative roll (bending into the minor groove) is plotted on the southern hemisphere of the dial. In the example shown, 30° roll and 20° tilt combine to give θ = 36° and ϕ = 34°

Some additional perspectives of the current study should be noted. Although the crystal structure data is well defined, the individual structures obtained for the crystalline state may be influenced significantly by packing effects (Dickerson et al., 1991; DiGabriele and Steitz, 1993; DiGabriele et al., 1989; Jain and Sundaralingam, 1989; Lipanov et al., 1993). The observation of bending predominantly at one end of the palindromic sequence d(CGCGAATTCGCG) (Dickerson et al., 1991) and of two structural forms for the same sequence d(CGCAAAAATGCG) in a single crystal (DiGabriele et al., 1989) underscores this problem. Dickerson and co-workers (Dickerson et al., 1994; Grzeskowiak et al., 1993) recently examined this issue and observed that sequence-specific base stacking interactions can be of significant magnitude to dominate local helical structure even within the restrictive environment of the crystal lattice.

Most recently, Dickerson et al. (1994) have surveyed packing effects in nine B-DNA dodecamers. They convincingly argue that intrinsic sequence effects establish the primary bending propensities and that packing effects influence the extent to which bending occurs or not in an individual crystal form. In particular, the GA step is identified as a flexible hinge that may be bent to various degrees by crystal forces as seen in the two ends of d(CGCGAATTCGCG). They emphasize the value of information from crystal structures on bendability. The intrinsic propensity for bending around the interfaces of the AT region with the CG flanking sequences in d(CGCGAATTCGCG) is supported by results from MD simulations (McConnell et al., 1994; Swaminathan et al., 1991), but the MD suggests that the position of each hinge region is distributed over at least two adjacent steps, rather than being localized to a single GA step.

The current study is focused on axis bending in oligonucleotides at the level of individual base pair steps. The 2 principal nucleotide base pairs A/T and G/C give rise to 16 possible base pair steps, only 10 of which are truly unique due to self-complementarity. The behavior of a given step may also be influenced by the adjacent steps on either side, leading to 136 unique tetrad possibilities when first neighbor context effects are considered (Yanagi et al., 1991). The crystallographic data base does not at this point contain examples of all tetrads, and thus a specific limitation of this study is the neglect of sequence context effects.

Finally, no attempt has been made to interpret the results on the basis of structural models (Hunter, 1993) or to incorporate the effect of thermal fluctuations on the structures in this study. One should also keep in mind that the diverse structures that serve as a database for this study were in fact refined to differing levels of resolution by many different groups, and thus are strictly comparable only within this limitation. These issues will be considered in more detail in a subsequent article in which bending dials from crystal structures are compared with structures obtained from MD simulations.

RESULTS

B-form structures

We first consider the bending observed in the 1334 base pair steps of 66 crystal structures of B-form DNA sequences (Table 2). We have omitted structures containing bases flipped over into a *syn* orientation of the χ torsion angle, as well as those structures co-crystallized with intercalating molecules. Some 23 of the B-form structures correspond to sequences complexed to nonintercalating drugs. The structures for protein-DNA complexes are analyzed separately (vide infra). Bending analysis of the B-form structures is shown in Fig. 2. The upper left hand corner of this figure is

TABLE 2 B-form DNA structures

NDB ID	Descriptor	Reference
BDBP23	5′-d(C(P^{Me})G)-3′	Han et al., 1990
BDFP24	5′-d(RG(P^{S})CG(P^{S})CG(P^{S})C)-3′	Cruse et al., 1986
BDJ008	5′-d(CCAAGATTGG)-3′	Prive et al., 1987
BDJ017	5′-d(CCAGGCCTGG)-3′	Heinemann and Alings, 1989
BDJ019	5′-d(CCAACGTTGG)-3′	Prive et al., 1991
BDJ025	5′-d(CGATCGATCG)-3′	Grzeskowiak et al., 1991
BDJ031	5′-d(CGATTAATCG)-3′	Quintana et al., 1992
BDJ036	5′-d(CGATATATCG)-3′, Ca^{2+}	Yuan et al., 1992
BDJ037	5′-d(CGATATATCG)-3′, Mg^{2+}	Yuan et al., 1992
BDJ039	5′-d(CCGGCGCCGG)-3′	Heinemann et al., 1992
BDJ045	5′-d(CCAAIATTGG)-3′	Lipanov et al.
BDJ051	5′-d(CATGGCCATG)-3′	Goodsell et al., 1993
BDJB27	5′-d(CCAGGC^{Me}CTGG)-3′	Heinemann and Hahn, 1992
BDJB43	5′-(CCAACITTGG)-3′, trigonal	Lipanov et al., 1993
BDJB44	5′-d(CCAACITTGG)-3′, monoclinic	Lipanov et al., 1993
BDJB48	5′-d(CGATCG^{Me}ATCG)-3′	Baikalov et al., 1993
BDJB49	5′-d(CCAGGC^{Me}CTGG)-3′, TNT refinement	Hahn and Heinemann, 1993
BDJB50	5′-d(CCAGGC^{Me}CTGG)-3′, X-PLOR refinement	Hahn and Heinemann, 1993
BDL001	5′-d(CGCGAATTCGCG)-3′, 290K	Drew et al., 1981
BDL002	5′-d(CGCGAATTCGCG)-3′, 16K	Drew et al., 1982
BDL005	5′-d(CGCGAATTCGCG)-3′, 290K	Holbrook et al., 1985
BDL006	5′-d(CGCAAAAAAGCG)-3′	Nelson et al., 1987
BDL007	5′-d(CGCATATATGCG)-3′	Yoon et al., 1988
BDL009	5′-d(CGCGAATTTGCG)-3′	Hunter et al., 1987b
BDL011	5′-d(CGCAAATTCGCG)-3′	Hunter et al., 1987a
BDL015	5′-d(CGCAAAAATGCG)-3′, up orientation	DiGabriele et al., 1989
BDL015	5′-d(CGCAAAAATGCG)-3′, down orientation	DiGabriele et al., 1989
BDL020	5′-d(CGCGAATTCGCG)-3′, re-refinement	Westhof, 1987
BDL021	5′-d(CGCGAAAACGCG)-3′/5′-d(CGCGTT) + (TTCGCG)-3′, model A4	Aymani et al., 1990
BDL028	5′-d(CGTGAATTCACG)-3′	Narayana et al., 1991
BLD029	5′-d(CGTGAATTCACG)-3′	Larsen et al., 1991
BDL032	5′-d(CGCGAAAACGCG)-3′/5′-d(CGCGTT) + (TTCGCG)-3′, model T4	Aymani et al., 1990
BDL038	5′-d(CGCAAATTTGCG)-3′	Edwards et al., 1992a
BDL042	5′-d(CGTAGATCTACG)-3′	Leonard and Hunter, 1993
BDL047	5′-d(CGCGAAAAAACG)-3′, orientation 1	DiGabriele and Steitz, 1993
BDL047	5′-d(CGCGAAAAAACG)-3′, orientation 2	DiGabriele and Steitz, 1993
BLD047	5′-d(CGCGAAAAAACG)-3′, orientation 3	DiGabriele and Steitz, 1993
BDLB03	5′-d(CGCGAATT^{Br}CGCG)-3′, MPD, 293K	Fratini et al., 1982
BDLB04	5′-d(CGCGAATT^{Br}CGCG)-3′, MPD, 280K	Fratini et al., 1982
BDLB13	5′-d(CGCGA^{Me}ATTCGCG)-3′	Frederick et al., 1988
BDLB26	5′-d(CGC^{Me}GAATTTGCG)-3′	Leonard et al., 1990
BDLB40	5′-d(CGCIAATTCGCG)-3′	Xuan and Weber, 1992
DDL017	5′-d(CGCGAATTCGCG)-3′, Cisplatin	Wing et al., 1984
GDL001	5′-d(CGCGATATCGCG)-3′, Netropsin, Conf. 1	Coll et al., 1989
GDL002	5′-d(CGCGAATTCGCG)-3′, Hoechst 33258	Teng et al., 1988
GDL003	5′-d(CGCAAATTTGCG)-3′, Distamycin	Coll et al., 1987
GDL004	5′-d(CGCGATATCGCG)-3′, Netropsin, Conf. 2	Coll et al., 1989
GDL006	5′-d(CGCGAATTCGCG)-3′, Hoechst 33258	Pjura et al., 1987
GDL008	5′-d(CGCGAATTCGCG)-3′, DAPI	Goodsell et al., 1989
GDL009	5′-d(CGCGAATTCGCG)-3′, Berenil	Brown et al., 1990
GDL010	5′-d(CGCGAATTCGCG)-3′, Hoechst 33258, 0°C, Piperazine Up	Quintana et al., 1991
GDL011	5′-d(CGCGAATTCGCG)-3′, Hoechst 33258, 0°C, Piperazine Down	Quintana et al., 1991
GDL012	5′-d(CGCGAATTCGCG)-3′, Hoechst 33258, −25°C, Piperazine Down	Quintana et al., 1991
GDL013	5′-d(CGCGAATTCGCG)-3′, Hoechst 33258, −100°C, Piperazine Down	Quintana et al., 1991
GDL014	5′-d(CGCAAATTTGCG)-3′, Netropsin	Tabernero et al., 1993
GDL015	5′-d(CGCGAATTCGCG)-3′, Pentamidine	Edwards et al., 1992b
GDL016	5′-d(CGCAAATTTGCG)-3′, Berenil	Brown et al., 1992
GDL018	5′-d(CGCGAATTCGCG)-3′, Netropsin	Sriram et al., 1992b
GDL021	5′-d(CGCGAATTCGCG)-3′, Hoechst 33342	Sriram et al., 1992a
GDL022	5′-d(CGCGAATTCGCG)-3′, Hoechst 33258	Sriram et al., 1992a
GDL023	5′-d(CGCGAATTCGCG)-3′, Propamidine	Nunn et al., 1993
GDLB05	5′-d(CGCGAATT^{Br}CGCG)-3′, Netropsin	Kopka et al., 1985
GDLB17	5′-d(CGC^{E}GAATTCGCG)-3′, Netropsin	Sriram et al., 1992b
GDLB19	5′-d(CGC^{E}GAATTCGCG)-3′, Hoechst 33258	Sriram et al., 1992a
GDLB20	5′-d(CGC^{E}GAATTCGCG)-3′, Hoechst 33342	Sriram et al., 1992a

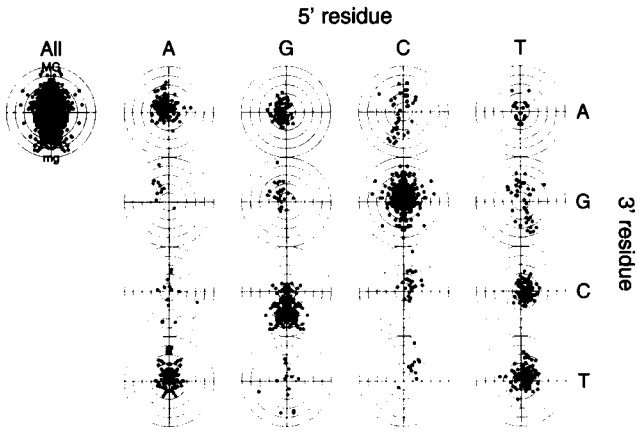

FIGURE 2 Analysis of local helix bending in 1334 base pair steps drawn from 66 different B-form DNA crystal structures currently resident in the NDB. The leftmost dial is the sum of all 1334 B-DNA steps resident in the NDB. This data is broken down into its 16 constituent two-base-pair steps in the remaining dials. Reading the sequence in the 5′ to 3′ direction, the steps are presented as a matrix in which the 5′ base is indicated by the column headings, and the 3′ base is read from the row headings. Bending compressing the major groove (MG) is in the north position on the dials, and bending into the opposing minor groove (mg) is in the south position. Each ring on a bending dial indicates a 5° deflection of the helical axis (θ), the maximum ring being 25°. Bending dials are defined in detail in the text and in Fig. 1.

a single bending dial containing a superposition of all the results. The major feature, echoing earlier analyses, is that bending is highly anisotropic. On the basis of the structures reported to date, bending into the major or minor grooves via base pair roll is preferred by a margin of ~2:1 over bending via tilt toward the sugar-phosphate backbones of the strands. Nevertheless, it must still be recognized that the results support a significant tilt component to bending and that it becomes more pronounced in structures with large bending magnitudes.

The remainder of Fig. 2 is a resolution of the bending analysis as described above into contributions from each base pair step. A matrix format is used for arrangement of the sixteen dials on the page, henceforth referred to as the bending dials matrix. All references to steps herein are in terms of the bases in the 5′ to 3′ direction, with the 5′ step read from the horizontal list and the 3′ step from the vertical. At the risk of introducing some redundancy, data points are included for both strands of the duplex. Thus, because of Watson-Crick base pairing, a palindromic step implies bilateral symmetry in the corresponding bending dial. Homopurine or homopyrimidine steps imply a symmetry relationship between two complementary dials in the matrix (for example AA and TT), having the same value of base pair roll but opposite in sign with respect to tilt. Each dial of course contains results from a particular step as found in diverse sequences. One point of note is the sparsity of data for a number of the cases; comments are kept to a minimum on cases with few data points, but it remains of interest to note emerging trends.

There is a particularly extensive data set for CG and GC steps. However, a number of these observations are from the various structures of the same sequence, the d(CGCGAATTCGCG) duplex, which is heavily represented at this time in the database. The bending dials for these steps, as shown in the bending dial matrix in Fig. 2, clearly exhibit preferential roll-bending into the minor groove for the GC step, with minor groove-directed bending seen in 170 of the 191 GC step examples (88%). Major groove-directed bending is distinctly evident for CG steps, seen in 229 of 357 of the CG step examples (63%). The AT, AG/CT, CG, AA/TT, and CC/GG steps all show preferential bending into the major groove. The CA/TG steps demonstrate a notable propensity to be bent in the direction of either the major or the minor groove.

Examining the patterns in bending with respect to τ, incipient anisotropy in tilt direction is seen in AA/TT, GA/TC, AG/CT, and GG/CC steps. The extent of bending with respect to τ, as noted above, is everywhere less than that with respect to ρ. Some emerging trends may be observed in the purine-purine and pyrimidine-pyrimidine steps, the upper left and lower right hand quadrants of the bending dial matrix in Fig. 2. These steps essentially all show indications of anisotropy in τ. Homopurine steps are observed to bend in the direction compressing their complementary Watson-Crick pyrimidine-pyrimidine sugar-phosphate backbone (the left hemisphere of the dial, indicating negative τ). The symmetry-related homopyrimidine steps thus bend into their own 5′-3′ sugar-phosphate backbone (right hemisphere, positive τ). For the most part, purine-purine and pyrimidine-pyrimidine steps additionally demonstrate a preference for bending in the direction of the major groove. The one exception to this major groove preference is found in the GA/TC steps, which show essentially isotropic bending with respect to ρ.

The bending dials matrix for the 23 drug-bound complexes of the B-form structures is shown in Fig. 3. No significant bending deviations from the above-mentioned trends can be discerned in the examples analyzed. Full reviews of crystal structures of drug-DNA complexes have been provided by Kennard (1993) and Zimmer and Wähnert (1986)

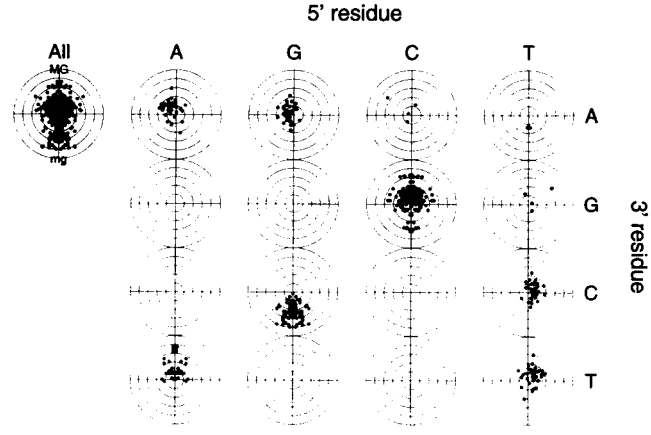

FIGURE 3 Analysis of local helix bending in the 486 steps in 23 crystal structures of B-form DNA-drug complexes currently resident in the NDB. See Fig. 2 for description.

Adenine tracts (A-tracts)

The bending dial analysis of observed bending in 15 B-form crystal structures containing stretches of three or more consecutive adenines on a strand is shown in Fig. 4. Bending at all AA steps within the A-tracts are denoted by filled circles and bending at steps within flanking sequences by open circles. The results, highlighted in Fig. 5, indicate that, within the A-tracts, the dispersion of bending values is less than that of the surrounding sequence and that A-tracts within oligonucleotide crystal structures are essentially straight, a result consistent with earlier A-tract crystal observations (DiGabriele and Steitz, 1993; Grzeskowiak et al., 1993). Systematic deviations from isotropic bending are particularly evident in steps flanking the A-tracts, consistent with the majority of the dodecamer structures crystallized in the same form of crystal lattice. This result is consistent with a simple junction bending model for A-tracts in the crystal, in which the localization of the A-tract bends are found outside of the A-tract regions as opposed to within.

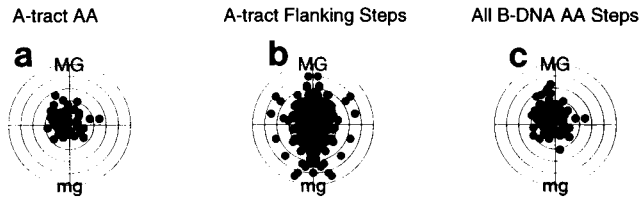

FIGURE 5 Overview of bending within the 15 A-tract structures analyzed in Fig. 4: (*a*) bending observed at the A-tract AA steps versus (*b*) bending in the steps flanking the A-tracts. (*c*) Bending observed in all B-DNA AA steps.

Dodecamers versus decamers: packing effects

Virtually all currently solved dodecamer oligonucleotide crystals have packed in the same orthorhombic form of lattice. In the organization of the unit cell, the helices are arranged such that the CG-rich flanking sequences overlap in rather close proximity, giving rise to helix-helix interactions that may influence helix bending. Recently, a total of 14 decamer oligonucleotides (see Table 2) were solved. Many of the DNA duplexes in these structures were observed to stack end to end and are free of at least one type of packing effect encountered in dodecamers. The results on axis bending in dodecamers and decamers are shown in Figs. 6 and 7.

Bending dial results for dodecamer and decamer structures are compared in Fig. 8. Fig. 8*a* contains the results for each

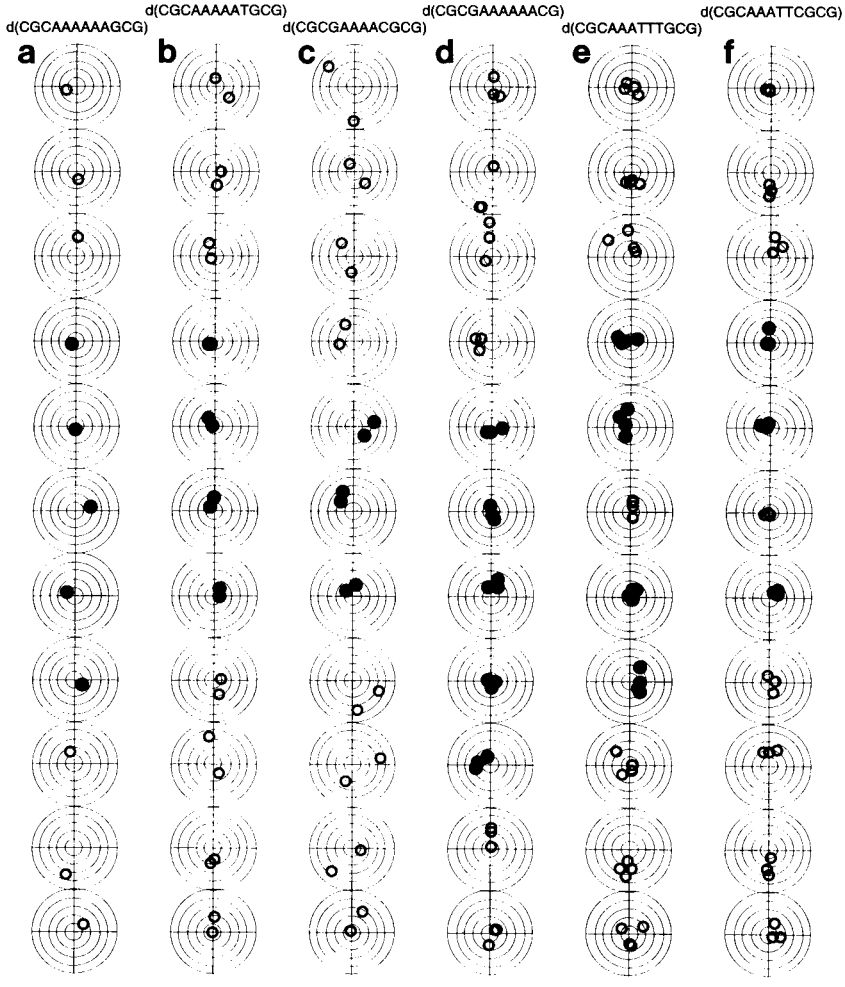

FIGURE 4 Stepwise analysis of bending in 15 examples of A-tract structures (defined by A_n, where n = 3) resident in the NDB. The filled circles indicate AA steps within the A-tracts, and the open circles indicate steps within the flanking sequences. The structures analyzed are: (*a*) d(CGCAAAAAAGCG) (Nelson et al., 1987); (*b*) d(CGCAAAAATGCG) (DiGabriele et al., 1989); (*c*) d(CGCGAAAACGCG) (Aymani et al., 1990); (*d*) d(CGCGAAAAAACG) (DiGabriele and Steitz, 1993); (*e*) d(CGCAAATTTGCG) (Brown et al., 1992; Coll et al., 1987; Edwards et al., 1992a; Tabernero et al., 1993); and (*f*) d(CGCAAATTCGCG) (Hunter et al., 1987a; Hunter et al., 1987b; Leonard et al., 1990). Note that, in most instances, more than one crystal structure has been determined for equivalent A-tract sequences, and this figure includes all available structures.

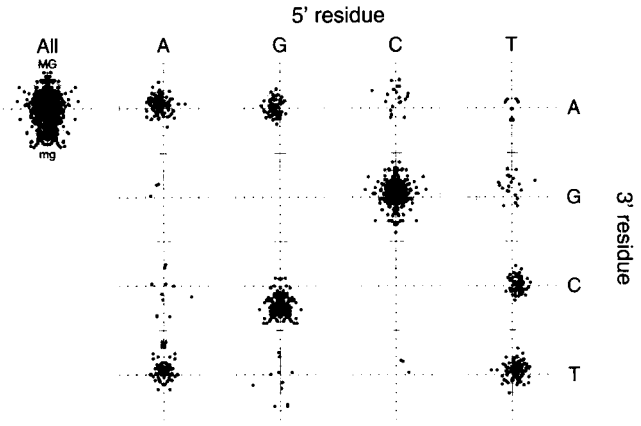

FIGURE 6 Bending dial matrix (see text) for crystal structures of B-form dodecamers.

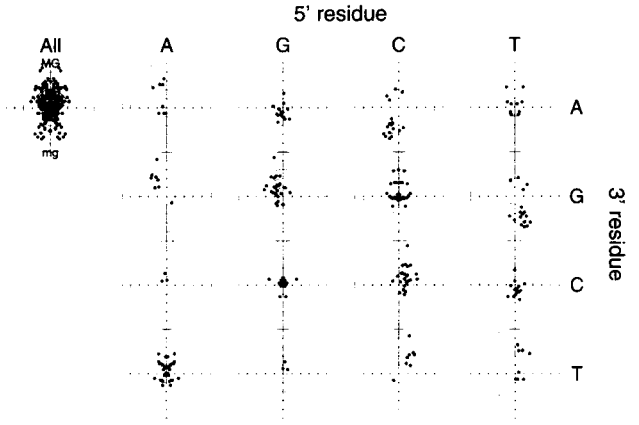

FIGURE 7 Bending dial matrix (see text) for crystal structures of B-form decamers.

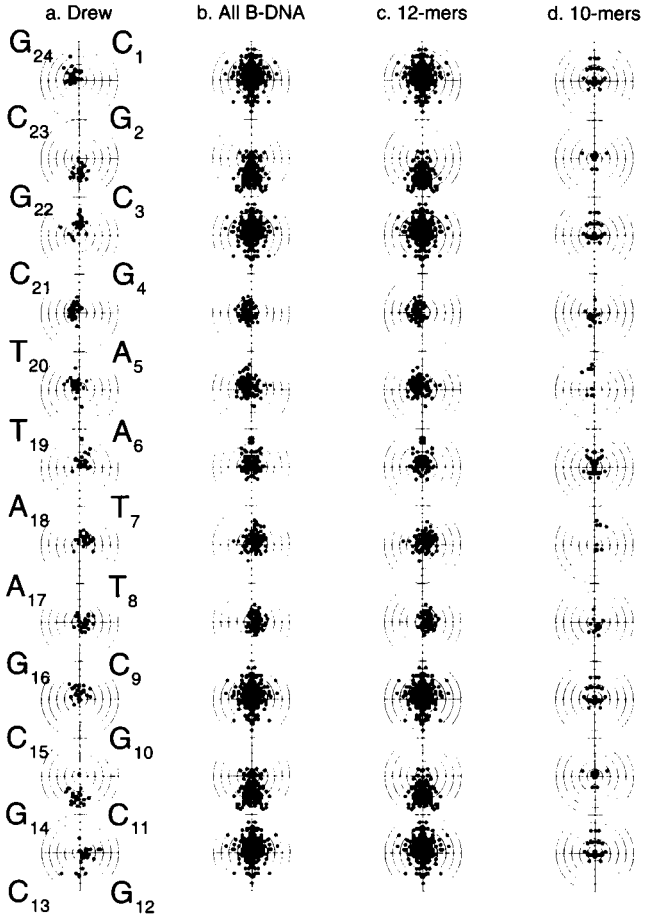

FIGURE 8 Calculated local axis bending for the sequence d(CGCGAATTCGCG) with corresponding results for all B-DNA structures, all dodecamers, and all decamers. (*a*) Bending dials calculated at each step in 25 homologous d(CGCGAATTCGCG) crystal structures. (*b*) Results for all appearances of each base pair step found in the sequence d(CGCGAATTCGCG) in all B-form structures in the NDB. (*c*) Results for all appearances of each step in all dodecamers in the NDB. (*d*) Results for all appearances in all decamer crystal stuctures in the NDB.

of the 11 base pair steps in the 25 structures reported to date for the d(CGCGAATTCGCG) duplex. Local bending occurs in all of these structures at two regions in the sequence, just adjacent to the interfaces of CG and AT regions. The dials indicate that the microscopic bend actually occurs over two base pair steps in each region, an outer step in which there is a bend toward the minor groove and an inner step in which there is a bend toward the major groove. The palindromic symmetry of the sequence is not precisely observed in the crystalline structure, and the regional bends do not turn out to be fully compensatory. Thus, a net overall bend occurs in the helix, as described in the original crystal structure analysis (Dickerson and Drew, 1981). Note that this precise description is calculated from roll and tilt as computed by the CURVES procedure, based upon the definition of a global helix axis. Thus, in this analysis, the flexible hinge region described recently by Dickerson et al. (1994) involves a region of several base pairs adjacent to the actual GA/TC junction, spanning from G_2-G_4 at the upper junction region and from C_9-C_{11} at the lower junction region.

In Fig. 8*b*, bending dials are shown for all constituent steps of the sequence d(CGCGAATTCGCG). These dials are composed of the sum of instances of these steps found anywhere in any B-form DNA sequence resident in the NDB. This column thus approximates generic behavior for the six distinct CG, GC, GA, AA, AT, and TC steps. The results, albeit heavily weighted by the presence of d(CGCGAATTCGCG) sequences (25 of the 66 B-DNA structural entries), fully support the bending pattern described above for the 25 crystallized examples of the d(CGCGAATTCGCG) sequence.

Fig. 8*c* shows the results drawn from all dodecamer structures in the NDB, all of course based on crystals of the same form. The results indicate that patterns observed in bending are not exclusively from the d(CGCGAATTCGCG) dodecamers but other dodecamer sequences as well.

Fig. 8*d* shows bending dials for all examples of the CG, GC, GA, AA, AT, and TC steps solved in decamer structures. The results clearly support the propensity for local bending into the major groove at CG steps. However, the tendency of GC steps to bend toward the minor groove in dodecamers is

not evident at the GC steps of the decamers. Particularly, every one of the GC steps observed in the 25 Drew sequence dodecamers (occurring at both step 2 and step 10) are seen to bend significantly into the minor groove, and fully 164 of 171 total GC steps occurring in isomorphous dodecamers bend into the minor groove. By contrast, 11 of 15 total GC steps found in decamers are seen to bend into the major groove, leaving only 4 of 15 (27%) bending into the minor groove. This indicates an area of axis bending in DNA dodecamers that is influenced by packing effects arising from helix-helix contacts (Dickerson et al., 1994; Narayana et al., 1991). This seemingly subtle manifestation of the crystal packing problem nevertheless has considerable import in using crystal structure data to critique results from theoretical simulations, to be discussed in a subsequent article.

Protein-DNA complexes

Protein-DNA crystal structures available for analysis are listed in Table 3. The DNA bending in 16 currently available protein-DNA complexes is shown in bending dial matrix form in Fig. 9. These results indicate that the majority of the local axis bends found in the protein-bound DNA helices are of roughly the same magnitude as the bends observed in the uncomplexed DNA structures. However, a small number of extreme cases of groove-directed local bending are noted, induced by protein-DNA complex formation. A deviation from uncomplexed B-DNA bending trends is seen in the reduced preference for groove-directed roll bends over backbone-directed tilt bends. Beyond the result that extreme kinks are a consequence of groove-directed local bends, there is surprisingly little evidence of systematic sequence effects in the results so far. The available data are too sparse for anything but provisional comment on this point. If this trend holds up, it may indicate that the DNA can be bent by a protein interaction at will. However, the observation of sequence-dependent binding specificity indicates otherwise. This situation is currently unresolved.

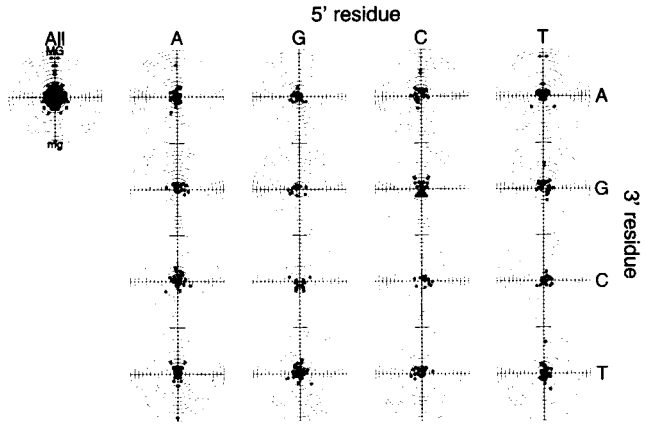

FIGURE 9 Analysis of 475 base pair steps crystallized in the 16 published DNA-protein complexes, presented as a bending dial matrix. Note that the scale of θ has been increased to 60° to accomodate all of the data points arising from sharp protein-induced kinks (each ring still represents a 5° axis deflection in θ).

A-form structures

The NDB now includes coordinates for 30 A-form DNA structures (Table 4). Helicoidal analysis was performed for these structures, and ROL and TLT values were used to compute θ and ϕ according to Eqs. 1 and 2. Note that our definition of bending is independent of helix type, as ROL and TLT are not features that discriminate between A- and B-form DNA. Canonical structures of both A- and B-form DNA have straight helical axis with ROL = TLT = 0 and are distinguished primarily by differences in displacement of bases and the inclination of the base pairs with respect to the helix axis (Ravishanker et al., 1989). The corresponding bending dials are shown in bending dial matrix form in Fig. 10. The global axis system utilized by the CURVES algorithm indicates a 20° inclination for canonical A-form DNA. However, roll and tilt values remain at 0°, placing the canonical points on the origin on the bending dials, just as in B-form DNA. The A-form structures do show a fair amount

TABLE 3 Protein-DNA complexes

NDB ID	Descriptor	Reference
PDE001	*Eco*RI endonuclease-DNA complex	Kim et al., 1990
PDE002	*Eco*RV endonuclease-DNA complex	Winkler et al., 1993
PDE003	*Eco*RV endonuclease-DNA complex	Winkler et al., 1993
PDE005	DNAse I-DNA complex	Weston et al., 1992
PDE006	DNAse I-DNA complex	Lahm and Suck, 1991
PDR001	434 Cro-DNA complex	Mondragon and Harrison, 1991
PDR004	434 repressor-operator complex	Aggarwal et al., 1988
PDR006	Catabolite gene activator-DNA complex	Schultz et al., 1991
PDR007	Lambda repressor-operator complex	Jordan and Pabo, 1988
PDR008	*E. coli* met repressor-operator complex	Somers and Phillips, 1992
PDR009	Trp repressor-operator complex	Otwinowski et al., 1988
PDR010	Lambda repressor-operator complex	Beamer and Pabo, 1992
PDR011	434 repressor-operator complex	Shimon and Harrison, 1993
PDRC01	Glucocorticoid receptor-DNA complex	Luisi et al., 1991
PDT004	Engrailed homeodomain-DNA complex	Kissinger et al., 1990
PDT006	Zif268-DNA complex	Pavletich and Pabo, 1991
PDV001	Bovine papillomavirus-1 E2-DNA complex	Hegde et al., 1992

TABLE 4 A-form DNA structures

NDB ID	Descriptor	Reference
ADDB01	5′-d((I)CCGG)-3′	Conner et al., 1984
ADH006	5′-d(GGGGCCCC)-3′	McCall et al., 1985
ADH007	5′-d(GGGATCCC)-3′	Lauble et al., 1988
ADH008	5′-d(GCCCGGGC)-3′	Heinemann et al., 1987
ADH012	5′-d(CCCCGGGG)-3′	Haran et al., 1987
ADH014	5′-d(GTGTACAC)-3′, spermine	Jain et al., 1989
ADH018	5′-d(GGGGTCCC)-3′	Kneale et al., 1985
ADH019	5′-d(GGGGCTCC)-3′	Hunter et al., 1986
ADH020	5′-d(CTCTAGAG)-3′	Hunter et al., 1989
ADH023	5′-d(GTACGTAC)-3′	Courseille et al., 1990
ADH024	5′-d(GTACGTAC)-3′	Takusagawa, 1990
ADH030	5′-d(GGGTACCC)-3′, room temperature	Eisenstein et al., 1990
ADH031	5′-d(GGGTACCC)-3′, 100K	Eisenstein et al., 1990
ADH038	5′-d(GTGTACAC)-3′, spermine	Thota et al., 1993
ADH039	5′-d(GTGTACAC)-3′, spermidine	Thota et al., 1993
ADH041	5′-d(GTCTAGAC)-3′	Cervi et al., 1992
ADHB11	5′-d(GG^{BR}UA^{Br}UACC)-3′	Kennard et al., 1986
ADHB17	5′-d(GGIGCTCC)-3′	Cruse et al., 1989
ADHP36	5′-d(GCCC(P^{Me})GGGC)-3′	Heinemann et al., 1991
ADI009	5′-d(GGATGGGAG)-3′	McCall et al., 1986
ADJ022	5′-d(ACCGGCCGGT)-3′	Frederick et al., 1989
ADJ050	5′-d(GCGGGCCCGC)-3′, orthorhombic	Ramakrishnan and Sundaralingam, 1993b
ADJ051	5′-d(GCGGGCCCGC)-3′, hexagonal	Ramakrishnan and Sundaralingam, 1993a
ADL025	5′-d(CCCCCGCGGGGG)-3′, spermine	Verdaguer et al., 1991
ADL045	5′-d(CCGTACGTACGG)-3′	Bingman et al., 1992b
ADL046	5′-d(GCGTACGTACGC)-3′	Bingman et al., 1992a
AHJ015	5′-r(GCG)-d(TATACGC)-3′	Wang et al., 1982
AHJ040	5′-d(GGGTATACGC)-3′/5′-r(GCG)-d(TATACCC)-3′	Egli et al., 1992
AHJ043	5′-r(G)-d(CGTATACGC)-3′	Egli et al., 1993
AHJ044	5′-d(GCGT)-r(A)-d(TACGC)-3′	Egli et al., 1993

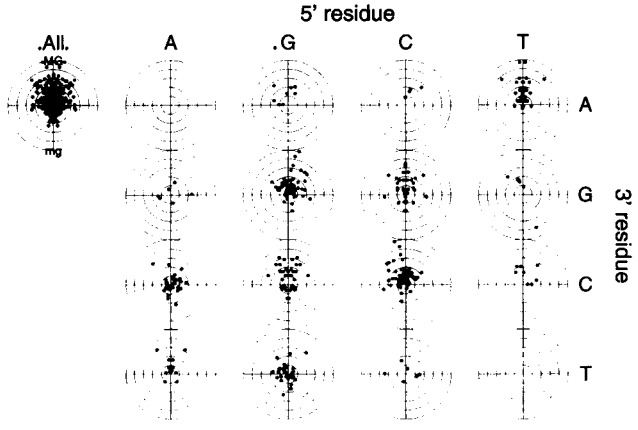

FIGURE 10 Bending analysis of 462 base pair steps found in all of the crystal structures of A-form DNA, presented as a bending dial matrix.

of concerted bending and particularly a tendency to bend via compression of the deep major groove rather than the more shallow minor groove. AC/GT steps have an elevated (37%) preference to bend in the direction of the minor groove, as compared with the 23% minor groove average for all A-form DNA steps. The limited data set relative to the crystallized B-DNA data set makes additional sequence-dependent analysis somewhat tenuous.

DISCUSSION

The results of Fig. 2 can be used to examine the extent to which trends predicted by Zhurkin and co-workers (Ulyanov and Zhurkin, 1984; Zhurkin, 1983; Zhurkin, 1985; Zhurkin et al., 1979, 1982) are followed in the current set of oligonucleotide crystal structures in the NDB. We find that in pyrimidine-purine steps (the four dials in the upper right hand block of Fig. 2) preferential bending toward the major groove is not exclusively observed. The highly represented CG step (356 instances) is the only pyrimidine-purine step to demonstrate any significant bias toward bending into the major groove. The CA/TG steps do not indicate a decided bending preference for either groove over the other but show large magnitude bends into both of the grooves. In the purine-pyrimidine steps (the four dials in the lower left hand block of Fig. 2), the GC step follows Zhurkin theory, bending almost exclusively into the minor groove. Bending at the AT step, on the other hand, is into the major groove in 91 of 123 cases (73%) and into the minor groove in the remaining 27%. Thus, the essential crystallographic evidence in support of the Zhurkin et al. original theory is the minor groove bending of the purine-pyrimidine GC step and the less definitive major groove bending observed in CG step data, although the more isotropic decamer subset of data for GC steps discussed above indicates that the data on GC steps may be influenced by packing effects.

Fig. 2 also facilitates an evaluation of the extent to which Dickerson's predictions of major groove bending LTP and minor groove bending HTP dimer step categories are represented in the current crystallographic database. Two homopurine steps, AA/TT and GG/CC, demonstrate a tendency to bend into the major groove, as predicted by their LTP classification. However, homopurine GA/TC steps do not

demonstrate the predicted major groove bending preference and are in fact nearly isotropic with respect to roll. Of the predicted minor groove bending HTP steps, AG/CT, AT, and CG, none demonstrate a definitive tendency to bend into the minor groove as predicted.

Dickerson noted earlier that there is no crystallographic evidence that suggests that A-tracts are curved within their length (Grzeskowiak et al., 1993). We find this observation remains valid for all 15 A-tract structures currently resident in the NDB and plotted in Fig. 4. The refinement of multiple orientations and geometries of chemically identical molecules within single crystal studies (DiGabriele and Steitz, 1993; DiGabriele et al., 1989) fueled concerns about the relative influence base pair sequence exerts over crystal packing effects in determining stepwise bending. In the same studies, however, Steitz observed that A-tracts are straight regardless of their relocation to inequivalent positions and orientations within the crystal lattice. When plotted within their respective molecular environments in Fig. 4, we see that, although some bending is observed at particular AA steps, the composite results are markedly isotropic in bending. The net results so far are consistent with effectively straight helix segments.

The junction model originally proposed by Crothers and co-workers (1990) was based essentially on changes in tilt or alternatively of inclination of base pairs with respect to the helix axis at GC/AT junctions. Dickerson et al. (1994) point out that, in the crystal structures they studied, bending at these junctions is produced by a pure roll motion. Examination of the bending dials in Fig. 4 shows that junction bending in d(CGCAAAAAAGCG) and d(CGCAAAAATGCG) are pure roll in nature whereas, in d(CGCGAAAACGCG), d(CGCGAAAAAACG), and d(CGCAAATTTGCG), one finds that both roll and tilt contribute to the observed bending. Note also that we find that the bending occurs not right at the junction but in the steps adjacent to the junction in the flanking sequence. Similar considerations apply to the bends in d(CGCGAATTCGCG). This leads us to the idea of a flexible hinge region, not a hinge confined to a single base pair step.

DNA bending resulting from isolated AA steps was recently hypothesized to be distinct from the bending effects of AA steps located within longer stretches of adenines, concluding that isolated AA steps do not confer additive bending effects when found within the double helix (Haran and Crothers, 1989). A comparison of AA step bending in the representative crystallographic A-tract structures (Fig. 5*a*) versus the sum total of all AA steps (Fig. 5*c*) reveals that it is difficult to distinguish the stepwise bending in the isolated AA occurrences from the AA bending observed within the 15 A-tract sequences. What is notable about the 15 representative A-tract structures is the relatively large magnitude θ values seen in the sequences flanking the A-tracts (Fig. 5*b*).

Extensions of the original pyrimidine-purine step theory have recently been described by Barber and Zhurkin (1990) in the analysis of a protein-DNA complex but are not necessarily applicable to uncomplexed forms. The analysis of the protein-DNA complexes suggests that groove-directed anisotropic bending is conserved within the extreme 30°-50° protein-induced local axis bends such as those observed in CAP (Schultz et al., 1991) where $(\theta_{max}, \phi) = (29°, 2°)$ and (33°, 359°), in the symmetrical *Eco*RI complex DNA (Kim et al., 1990) where $(\theta_{max}, \phi) = (40°, 356°)$ and (57°, 180°), and also in the *Eco*RV complex (Winkler et al., 1993) where $(\theta_{max}, \phi) = (50°, 8°)$. Fig. 11 presents an overview of the bending observed in all of the subgroups investigated in this study, highlighting the intensity of the extreme local bends that can be induced by DNA-binding proteins.

Although it is easy to be drawn to the extreme examples of kinked localized bending noted above, the analysis on protein-DNA complexes shows that it is also possible for DNA to interact with proteins solely through a more gradual additive bend, as is observed in the 35° overall bend (calculated by using the persistence algorithm (Prevost et al., 1993)) seen in the phage 434 repressor/DNA complex (Aggarwal et al., 1988), which is gently introduced over 18 base pair steps with no individual step θ being greater than 21°. Results in Fig. 9 suggest that the presence of a bound protein about a potentially malleable DNA helix seems capable of imparting sufficient influence onto the DNA molecule to largely negate the 5°-15° sequence-dependent bending preferences noted in Fig. 2 for uncomplexed DNA. It remains to be seen whether the energetically (and potentially biologically) significant DNA bending preferences may indeed be found in that small number of extreme kink-type bends that

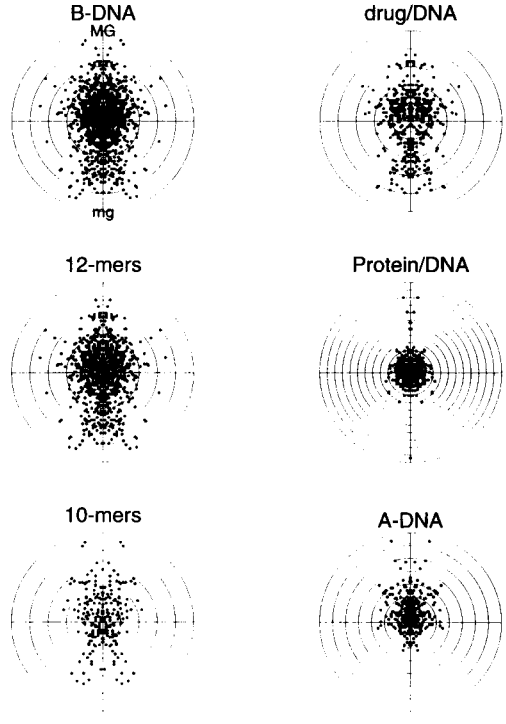

FIGURE 11 Collective results for local axis bending for the major categories of DNA x-ray crystal structures published to date, presented as bending dials. In all plots, each concentric ring indicates a 5° increment in the magnitude of axis deflection, θ.

lie outside of the realm of the 5°-15° bending preferences observed in the unbound DNA in Fig. 2.

While this article was in the final stages of reviewing, Goodsell and Dickerson (1994) have reported an alternative approach to bending and curvature calculation in B-form DNA, by using a graphical tool similar to that proposed earlier by Prevost et al. (1993); cf. Fig. 2 of Goodsell et al., 1994, and Figs. 15 and 16 of Prevost et al., 1993. The procedure was used to evaluate macroscopic curvature resulting from six different DNA bending models composed of widely different contributions from TWS, ROL, and TLT. All turned out to be fairly consistent with experimental data on gel retardation and cyclization kinetics on A-tracts. The author's nucleosome positioning model involves local bending at mixed sequence DNA with strong bending at GGC triads and straight, rigid A-tracts as evidenced by the crystal structure results. Our analysis of dimer steps, in addition to providing additional evidence for straight A-tracts, indicates (cf. Fig. 2) that GC steps strongly favor bending into the minor groove and that GG steps show a preference for bending into the major groove, but the data on this case are sparse so far.

SUMMARY AND CONCLUSIONS

In summary, patterns in dinucleotide base step bending have been analyzed in 66 examples of B-form DNA, 30 examples of A-form DNA, and 16 examples of B-form DNA complexed to DNA-binding proteins. Composite results from structures in these various categories are presented as bending dials in Fig. 11. Significant sequence-dependent bending characteristics are supported by the available data. This analysis serves as a catalogue of crystallographically observed sequence-dependent bending available up to this point, and represents the complete known spectrum of local bending at the base step level of the DNA molecule found in a crystal environment. A better understanding of the nature of DNA bending and bendability should result from maintaining the analysis as described herein as additional crystal structures of oligonucleotides sequences in free and bound forms are reported. In a subsequent article, we will report the bending characteristics of dynamical models of oligonucleotides obtained from molecular simulations and use bending dials as the basis for a comparison of calculated and observed results.

Conversations and advice from Dr. Richard Lavery and Prof. Stephen Harvey on the calculation and presentation of bending dials are gratefully acknowledged. The authors particularly wish to acknowledge preliminary studies to this project carried out by Mr. Michael J. Censullo in our laboratory and reported in his M.A. thesis (Wesleyan University, 1990).
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