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Molecular Dynamics Simulations of DNA Curvature and Flexibility: Helix Phasing and Premelting

Abstract: *Recent studies of DNA axis curvature and flexibility based on molecular dynamics (MD) simulations on DNA are reviewed. The MD simulations are on DNA sequences up to 25 base pairs in length, including explicit consideration of counterions and waters in the computational model. MD studies are described for ApA steps, A-tracts, for sequences of A-tracts with helix phasing. In MD modeling, ApA steps and A-tracts in aqueous solution are essentially straight, relatively rigid, and exhibit the characteristic features associated with the B′-form of DNA. The results of MD modeling of A-tract oligonucleotides are validated by close accord with corresponding crystal structure results and nuclear magnetic resonance (NMR) nuclear Overhauser effect (NOE) and residual dipolar coupling (RDC) structures of d(CGCGAATTCGCG) and d(GGCAAAAAACGG). MD simulation successfully accounts for enhanced axis curvature in a set of three sequences with phased A-tracts studied to date. The primary origin of the axis curvature in the MD model is found at those pyrimidine/purine YpR "flexible hinge points" in a high* roll, *open hinge conformational substate. In the MD model of axis curvature in a DNA sequence with both phased A-tracts and YpR steps, the A-tracts appear to act as* positioning elements *that make the helix phasing more precise, and key YpR steps in the open hinge state serve as* curvature elements. *Our simulations on a phased A-tract sequence as a function of temperature show that the MD simulations exhibit a premelting transition in close accord with experiment, and predict that the mechanism involves a B′-to-B transition within A-tracts coupled with the prediction of a transition in key YpR steps from the high roll, open hinge, to a low roll, closed hinge substate. Diverse experimental observations on DNA curvature phenomena are examined in light of the MD model with no serious discrepancies. The collected MD results provide independent support for the "non-A-tract model" of DNA curvature. The "junction model" is indicated to be a special case of the non-A-tract model when there is a Y base at the 5′ end of an A-tract. In accord with crystallography, the "ApA wedge model" is not supported by MD.* © 2004 Wiley Periodicals, Inc. Biopolymers 73: 380–403, 2004

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INTRODUCTION

DNA sequence-dependent axis curvature and flexibility are phenomena of particular importance in understanding the structural biology of DNA and protein–DNA interactions.[1–4] A key concept in this area of research is the phenomenon of "helix phasing", in which particular base pair sequence elements phased by an integral number of turns in a B-form DNA helix give rise to anomalously high results in diverse empirical measures of axis curvature.[5] The structural origin of the helix phasing phenomena is at a series of local deformations all on the same side of the double helix. This serves to amplify the effect of any one in a concerted manner. However, the nature of the local deformations involved and the details of the helix-phasing phenomenon at the molecular level remain an active area of research. Understanding DNA curvature and flexibility, and helix phasing in particular, carries considerable implications with respect to diverse questions ranging from the nature of nucleosome structure[6] to the fundamental aspects of genetic recognition at the molecular level.[4]

The salient issues currently before us in DNA curvature are the nature of structural motifs responsible for the local sequence deformations and the details of how they operate cooperatively to bring about the phenomena. The prototype and the most widely studied case of the helix phasing is phased "A-tracts," i.e., a contiguous series of three or more ApA steps, linked in diverse experiments to the enhancement of DNA curvature.[5,7] Unsettled questions include the molecular structures of ApA steps and A-tracts in solution, and the nature of the stabilizing forces involved, as well as the detailed molecular mechanism involved in helix phasing. The evidence that A-tracts are unique comes from the fiber diffraction and crystallography[8] and the presence of "premelting transition" in AT-rich DNA sequences.[9] The influence of hydration and the possible participation of transient complexes involving mobile counterions in DNA curvature phenomena[10–16] remain to be fully clarified. The idea of pyrimidine–purine (YpR) steps as a primary source of DNA deformation emerged from early theoretical studies.[17] The YpR motif is ubiquitous in protein-induced DNA bending based on the crystal structures of many protein–DNA complexes[18,19]; thus, a role for YpR steps in the curvature of uncomplexed DNA must be considered a serious possibility as well. In contrast, the evidence that phased A-tracts are linked to DNA curvature is unequivocal.[2,5,20] However, if a non-A-tract, YpR kink is the origin of intrinsic curvature (instead of an ApA step, an A-tract, or the junction of A-tracts with flanking sequence elements), the question of exactly how phased A-tracts contribute is raised. Also, the helix phasing phenomena is not limited only to A-tracts as the operational sequence motif,[16,21] and there is considerable interest in knowing the general characteristics required of helix phasing elements.[6]

Experimental techniques for all-atom nucleic acid structure determination using crystallography or nuclear magnetic resonance (NMR) spectroscopy have served as a basis for major contributions to DNA structure at the oligonucleotide level, but are not yet capable of direct study of sequences of the lengths of ~30 base pairs (bp) in which a prototype helix phasing is presented. As a consequence, critical aspects of the helix phasing problem at the molecular level are still at the level of conjecture and somewhat controversial. Recently, with theoretical and methodological developments in molecular simulation and technological developments in high-performance computing, it has become possible to obtain highly detailed all-atom computational models of DNA oligonucleotides of intermediate length in aqueous solution, using molecular dynamics (MD) computer simulation. With an MD model that successfully describes DNA structure in solution, one can, in principle, perform studies directly on helix-phasing sequences of different compositions, examine the contributing structures using computer graphics, and obtain a description of sequence-dependent DNA curvature and flexibility. The MD model in this case is an essentially ab initio result, with no details of axis curvature per se introduced into the parameterization of the MD force field. The MD results can then serve as a basis for evaluating the viability of various models proposed ad hoc to explain the phenomenon. At this point, only a small number of MD studies of DNA curvature have been reported, but the results are found to successfully integrate a number of the disparate ideas in the field and offer a possible resolution of a number of outstanding research questions.

In this article, we review the results obtained to date from MD studies of the intrinsic curvature and flexibility of DNA oligonucleotides free in solution. As a point of validation, we also review recent studies of how well MD on DNA agrees with experimental data; describe recent results from MD on the dynam-

ical structure of ApA steps, A-tracts, and A-tracts in aqueous solution; and then survey recent results on sequences with phased A-tracts and the closely related phenomenon of the "premelting transition" in DNA.

We note in passing that general accord in the field as to the use of various terms describing DNA deformations has not yet been established. In this article, DNA *deformation* will be used as a generic term for any local or global deviations from specified monotonous reference structures, such as the canonical A- and B-forms of DNA identified in fiber diffraction studies. The dy*namical structure* of a DNA oligonucleotide is defined as the ensemble of instantaneous structures (I-structures[22]) that comprise a Boltzmann distribution at a specific temperature. Dynamical structure is the most rigorous way to think about any molecule or macromolecule, DNA included, from the point of view of statistical mechanics. Following particularly Hagerman[5] and Maher,[23] the term "DNA *curvature*" will be used exclusively to refer to the intrinsic deformation of a DNA sequence globally or locally. "DNA *flexibility*" will be used to refer to the sequence-dependent thermal fluctuations in the structure. Diverse methods have been proposed for calculating the curvature of any single DNA snapshot or part thereof, but there is no unique method that produces a well-defined, observable expectation value. However, once a definition is specified, both DNA curvature and flexibility can readily be calculated from MD as a property of the full ensemble or substates thereof, should they be relevant. We shall use the term "DNA *bending*" to refer the alteration of DNA structure in complexes with proteins or other ligands. For a DNA sequence in a complex, bending arises as consequence of the intrinsic curvature, as defined above, and the protein induced deformations. "DNA *bendability*" will be used to refer to the sequence-dependent susceptibility of a structure to ligand-induced deformations. Thus, as used herein, DNA *flexibility* and bendability refer to intrinsic and ligand-induced dynamics, respectively. Finally, we along with others, taking a statistical mechanics view of the problem,[5,24–26] have noted that the very idea of a "static structure" for a DNA molecule in solution is highly problematic. Whether any of the single I-structure snapshots from MD, or indeed any average structure defined on a Boltzmann ensemble of DNA structures, is in any way representative of the properly defined dynamical structure of DNA at a given temperature and set of environmental conditions cannot be safely assumed, since it is likely that substates are involved to some extent. For the present purposes, to differentiate any single structure representation from a dynamical all-atom structure of DNA, any single "static" structure for a DNA molecule will be referred to as a "structural construct."[27] The place of a static structure of DNA in interpreting experimental results has, however, been debated.[28]

In the preparation of this article, we are indebted to previous reviews on DNA curvature and bending by Sundaralingam and Sekharudu,[29] Hagerman,[5] Olson and Zhurkin,[26] Crothers and Shakked,[30] and Hud and Plavec.[16] A general perspective on the subject and the related, even more fundamental, issue of DNA sequence effects on structure is provided in the texts by Calladine and Drew,[3] Sinden,[2] Neidle,[4] and the review article by Lavery and Zakrezewska.[31]

BACKGROUND

The 50th anniversary of the discovery of the structure of DNA as the double helix is being widely celebrated this year as one of the most momentous scientific discoveries of the twentieth century.[32,33] The biological implications of DNA structure, i.e., that B-DNA is predominant in cellular conditions, that DNA has the capacity for self-replication, and that the sequence of nucleotide bases codes for the structure of proteins, followed in a dramatic series of subsequent discoveries.[34] A wide range of experimental and theoretical methods have now been applied to DNA to elucidate further details at the molecular and atomic level. The atomic coordinates of an average or canonical structure of the Watson–Crick double helix, which has come to be known as the B-form of DNA,[35] were derived from fiber diffraction experiments.[36] The families of DNA structures identified from fiber diffraction and crystallography are denoted A, A′, B, B′ C, D,. . .,Z.[37,38] Selected DNA structures relevant to this article are shown in Figure 1. The first crystal structure of a B-form DNA, considered the predominant structural form in vivo, was reported in 1981 by Dickerson and coworkers.[39–41] This structure, of sequence d(CGCGAATTCGCG), provided the first glimpse of sequence-dependent axis deformations of DNA at the molecular level. With regard to the structure of DNA in solution, the full determination of high-resolution all-atom models was found to be beyond the range of NMR nuclear Overhauser effect (NOE) and spin-coupling experiments, although identification of helix type and general structural features proved quite feasible.[42] Recently, new NMR methods have been developed based on residual dipolar coupling (RDC) experiments, which can resolve the helicoidal parameters of DNA in solution more accurately.[43,44] RDC structures of oligonucleotides are just beginning to appear.[43–46] In particular, the RDC tech-

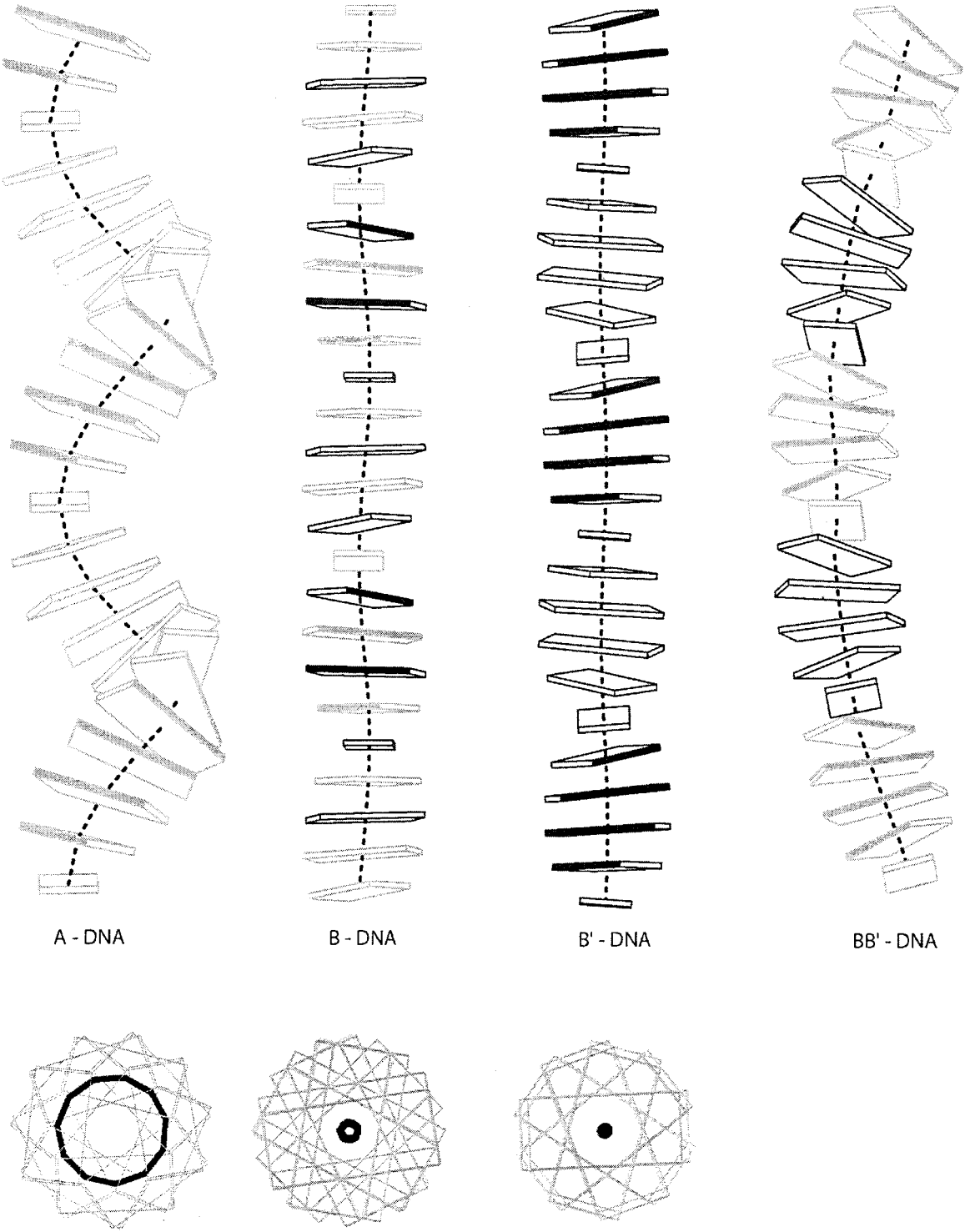

FIGURE 1 Canonical forms of A, B, B′ and mixed B/B′ DNA, with the path taken by the central axis shown with a dark dotted line. The lower part of the figure shows the corresponding view down the helical axis for the A, B, and B′ DNA. The central circle in each of these images shows the local curvature as a result of writhe, which also shows up in a normal vector plot, although the global axis is straight. The A-DNA structure forms an approximate ring in the normal vector plot with the largest radii followed by B′ and B DNA. The pure B′ form DNA has the straight global axis. All DNA structures are based on the Arnott et al.[36] parameters and have been generated using the X3DNA program developed by Lu et al.[181]

nique has been applied to A-tracts,[45,46] but not to phased A-tracts. The results on short oligonucleotides relevant to DNA curvature will be discussed below.

The crystallographic databases[47,48] serve as the primary source of independent experimental data on dinucleotide step parameters relevant to axis curvature and flexibility. There are 16 total XpY step permutations for A, T, G, and C bases, of which 10 are unique. The crystallographic database remains heavily biased toward cases with G and C. Crystal structures are certainly sensitive to regions of a crystal structure in which there are local helix–helix contacts, such as the major groove G-G clashes in d(CGCGAATTCGCG) oligonucleotides that crystallize in $P2_12_12_1$ space groups. There are now several notable cases of crystals with more than one DNA molecule per asymmetric unit that differ in axis curvature,[49,50] so this property is undoubtedly sensitive to crystal packing effects.[51] The recent NMR structure of d(GGCAAAAAACGG) in solution[45] differs with the corresponding crystal structure[52] on the direction of curvature. However, direction of curvature is more subtle than curvature per se or sequence effects in general. The view on DNA sequence effects from crystallography is the most robust to date and, considered in context, is not to be dismissed lightly. However, concerns have been raised in particular about the possible sensitivity of ApA steps, a key sequence motif in DNA curvature, to environmental effects.[53]

Another problem is that of sequence context effects. If the structure of any individual XpY step is subject to nearest neighbors, a trimeric (32 unique permutations), or even a tetradic (136 unique permutations) unit, may be necessary to serve as a suitable building block for higher-order structures. Sets of data indexing structures with respect to trinucleotide steps have been derived from DNase digestion[54,55] and nucleosome positioning,[56] respectively. Both sets of results indicate significant context effects for dinucleotide steps. However, the rankings do not correlate well with each other, and thus must relate to different aspects of the phenomena that are not yet well resolved. At the tetranucleotide step level, the crystallographic database is sparse, although sufficient to signal the potential seriousness of the preferential stability of different substates in different sequence contexts.[3] An extensive theoretical consideration of the problem has been provided based on stacking energy calculations.[57,58] Although the model used in these studies has been criticized based on quantum chemistry,[59] still many useful hypotheses about the nature of sequence effects were advanced in this work, and provocative questions for further studies were well posed.

At this point in time, crystal structures of a number of B-form DNA oligonucleotides have been examined, the conformational and helicoidal parameters[31] calculated, and the trends and patterns in the results with respect to sequence have been examined.[3,60–64] A current data set is available at http://rutchem.rutgers.edu/~olson/pdna.html. The DNA dinucleotide steps may be grouped into the classes YpR, YpY (=RpR) and RpY, where Y stands for pyrimidine bases and R for purines on a single strand of a duplex reading 5′ to 3′. With respect to molecular geometry, the problem can be reduced to the base pair step helicoidal parameters that show significant variability over the data base: *roll, tilt, twist, shift, slide,* and *rise.* (Italics indicate parameters defined according to the currently observed "Cambridge" naming convention[65,66].) The YpR steps were identified in early energy function calculations and Monte Carlo simulations by Zhurkin and coworkers[17,24] as sites that exhibit the most flexibility and extreme local deformations in B-form DNA, characterized particularly by a positive values of *roll,* i.e., a local helix deformation toward, and thus narrowing the major groove. Not all YpR steps are deformed, but all appear to exhibit a susceptibility to deformation, and have been described as "flexible hinges"[3] due to evidence of context-dependent bistability. Yanagi et al.[61] stressed the flexibility of CpA steps in their retrospective analysis of crystal structure data ca. 1991, and categorized the structures into high *twist* profile (HTP) with low *roll* and low *twist* profile (LTP) with higher *roll* values. Experimental support for this comes from the work of Beutel and Gold[67] and Nagaich et al.[68] Goodsell et al.[69] have noted the implications of TpA steps in DNA curvature. Flexibility in CpG steps has been noted in NMR studies by Lefebvre et al.[70–72] The potential bistability of YpR steps has been further elucidated in the theoretical analyses of Hunter[73] and discussed by Calladine and Drew.[3] The idea is that for YpR steps there are two substates, one showing low *slide* and low *roll,* as in B-form DNA, and negative *slide* and positive *roll,* toward the values associated more typical of A-form structures, noted to be isomorphous with the HTP and LTP categories by Hunter [73]. We will henceforth refer to the substates of YpR steps as the "closed hinge" and "open hinge" forms, respectively, noting that the hinge motion may involve cooperative changes in several helicoidal variables. The most recent survey of the crystal structures of uncomplexed DNA oligonucleotides (Olson et al.; web site noted above) supports this idea more emphatically for TpA and CpA (=TpG) steps than for CpG. Of course, there is no guarantee that all substates are, or should be, represented in the crystal structures to date. The RpY steps

in B-form DNA favor deformations of *roll* toward and subsequently narrowing the minor groove, but not so extreme as those of some YpR steps toward the major grove. The deformations at RpR steps are less than at YpR steps, are similar in range to RpY steps, and descend from positive *roll* at ApG and GpG, with ApA near zero in *roll* and GpA showing, on the average, negative *roll.* In considering these trends, it is important to note that statistical uncertainties as reflected in the standard deviations of the means for helicoidal parameters derived from the crystal structure data are typically large (for a recent listing, see Liu and Beveridge[27]).

The earliest considerations of DNA deformation as a factor to contend with in the structural biology of DNA emerged from studies on the nucleosome[74,75]; nucleosome structure and dynamics remain an active area of research.[6,76] Early statistical analysis of DNA sequences revealed that nucleosomal DNA, which must be curved for compaction, features ApA steps spaced by a full turn of a B-form helix.[77,78] Experimental evidence accumulated that certain DNA sequences were associated with anomalous gel mobility and facilitate the formation of DNA minicircles. On the basis of birefrigence experiments, Hagerman[79] provided evidence for stable curvature of DNA in solution and that gel retardation was not just a consequence of DNA flexibility. Hagerman[20] also presented in vitro validation of the helix-phasing hypothesis based on A-tracts. The length dependence of cyclization assays on phased A-tracts was determined to be optimum for ~126 bp,[80,81] which resulted in a net curvature of $-8.7°$ per ApA step, assuming this to be the operational element. Koo and Crothers[82] determined that the direction of overall curvature of a sequence with phased A-tracts is toward the minor groove of the DNA with respect to the central A-tracts. The extent of curvature was determined by Koo et al.[83] to be 17–21° per A-tract for phased A_6 motifs (strictly speaking, per monomeric element). A recent topological measurement confirms this,[84] and the structural implication of this result is discussed later in this article. Hagerman's observation that phased A_4T_4 motifs show enhanced curvature, whereas phased T_4A_4 motifs do not, has pointed to the effect of 5′–3′ sequence polarity on the phasing phenomena[85] and calls attention to the TpA step.[24,69]

Biophysical studies of DNA curvature in solution have used diverse methods,[5,23,86] but all result in one, or at most a few, metrics per sequence, leaving the all-atom sequence-dependent structure of the double helix highly underdetermined. Each individual type of experiment measures something a little different from all the others, and this is difficult to translate precisely into structure. For example, gel retardation reflects a tendency of a DNA sequence to straighten and pass through pores in a gel, while cyclization assays reflect the tendency of a sequence to adopt planar curved structures. Structures contributing to these phenomena come from different regions of the Boltzmann distribution, and thus neither gel retardation nor cyclization assays reflects the equilibrium dynamical structure of a sequence. New spectroscopic methods, such as Fluorescence Resonance Energy Transfer (FRET),[87] Lenthenite Resonance Energy Transfer (LRET),[88] and Transient Electric Birefringance (TEB)[89] are being applied to this problem. A comprehensive database of spectroscopic results on curvature has not yet been assembled, but results to date are promising, and these techniques are being widely adopted.[90] In the analysis of DNA curvature, the results of the various biophysical measurements are typically reduced to retardation constant R_L[91] or an effective curvature angle α.[92] These indices either implicitly or explicitly assume a simple, unidirectional model of curvature, a highly oversimplified view of the way a particular flexible DNA sequence actually curves and kinks in a sample. However, novel research designs based on ligand ladders[21,23] has led to characterization of quite subtle aspects of the helix-phasing phenomena.

The collected data on effective curvature as a function of DNA sequence have served as a basis for deriving dinucleotide step parameters.[27,91,93–95] The various molecular models proposed to date to explain DNA curvature are shown schematically in Figure 2, adapted from Goodsell et al.[96] The various molecular models differ with respect to whether the main origin of axis curvature in sequences of phased A-tracts is found within A-tracts (the ApA wedge model[77,91]) (Figure 2a), at the interface of essentially straight A-tracts and flanking sequences (the junction model[82,97]) (Figure 2b), or in regions of the sequence other than A-tracts (the non-A-tract model) (Figure 2c). The non-A-tract model, following earlier steps clearly in this direction[24,52,98,99] was given full articulation by Dickerson and coworkers.[96]

A key early work providing support for the wedge model is that of Bolshoy et al.,[91] who carried out regression analysis of a sizable database of R_L values against *roll, tilt,* and *twist* by step. The ApA *roll* angle of ~8.7° derived by Ulanovsky et al.[80,81], was a key feature in the initialization of the analysis, and the regression converged to a model retaining this large ApA wedge angle, which implies "bent A-tracts." This model of DNA curvature conflicts with the structures of ApA steps in all A-tracts from crystallography[8] and raised the possibility that the crystal struc-

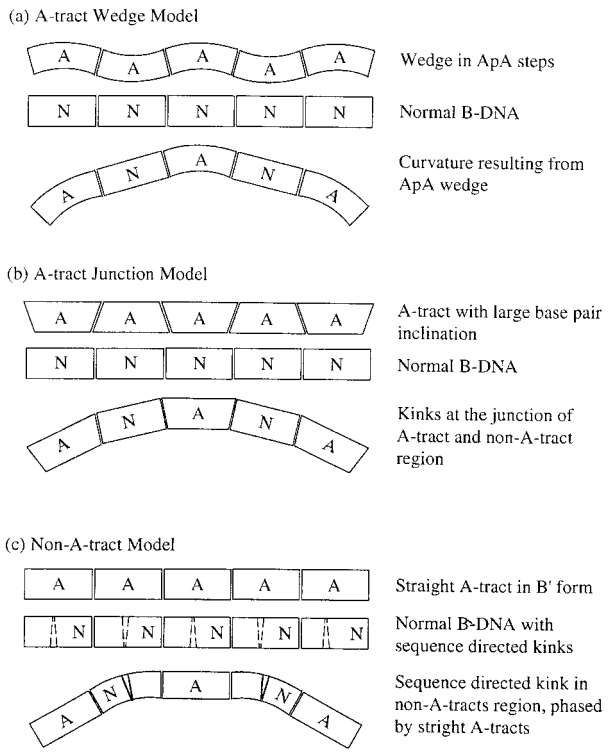

FIGURE 2 Schematic diagram of the principle molecular models for DNA curvature in phased A-tracts, adapted and redrawn from Goodsell et al.[69]

ture results might not be a reliable indicator of DNA structure in solution.[53,100] This issue has become somewhat of an enduring controversy in the field. However, Hagerman[85] reported early on that electrophoretic analysis indicated that a wedge model does not hold up in a critical test of the idea based on solution phase experimental data. A novel role for flexibility in DNA bending was emphasized particularly in the "flexible wedge" model of Olson et al.,[25] which suggested that the discrepancy between crystallographic ApA steps (straight) and the ApA wedge (bent) model for the solution structure might reside in the thermal population of bent forms at high temperature on an asymmetric potential that has a minimum (and therefore crystal structure) at a straight B-form geometry.

The idea of a junction model originates in the deviation in the trajectory of a (locally defined) helix axis when two sequence elements with different base pair inclination are juxtaposed so as to maintain maximal base pair stacking. A classic example is the A/B junction.[101] Crothers and coworkers[82,97] have made extensive interpretation of data for DNA curvature based on variations of this model with either base pair *tilt* or *inclination* as the operational factor at the point of articulation. However, these investigators have been careful to point out that the curvature of 17–21° produced in a sequence of phased A6 elements does not distinguish among proposed models, since any model that produces this overall bend is consistent with the result,[83] a point reiterated by Crothers and Drak.[102] Levene and Crothers[93] proposed a method of obtaining structures from cyclization data by Monte Carlo methods, essentially a scan of combinations of parameters of a reduced description of DNA, interpreted in terms of a junction model. An argument against the junction model is that crystal structure results on oligonucleotide sequence in which A-tracts and non-A-tract elements abut do not generally show a localized kink at the interface or a junction model of curvature.[8,96] Also the determination of base pair step parameters by numerical fits to the collected data to date (see above) do not converge to a junction bend model. In more recent accounts, this model is evolving in the direction of delocalized curvature[46] (see also Strahs and Schlick[103]).

The non-A-tract model is based on the idea that A-tracts in solution as observed in crystals are rigid and essentially straight, and that the origin of DNA curvature is in the non-A-tract sequence elements flanking the A-tract. The nature of this curvature has been discussed as uncompensated writhe[98] or as YpR kinks.[24,96,98,99] The argument against the non-A-tract model is that the crystal structure results may not be a good predictor of structure in solution, and that if A-tracts are straight, the structural parameters of the B-form DNA in the non-A-tract region would require a significant revision in the structure of generic or canonical B-DNA, since high inclination and roll would be required to account for the measured 17–21° angle of curvature.[104] A third point is the "contradiction in terms" that A-tract-induced curvature does not originate in A-tracts! However, the link between anomalous indices of DNA curvature and phased A-tracts is quite secure, raising the question of the precise role of the A-tracts.

Integrative studies of the ability of the various models of this genre to account for the observed data have been provided by Tan and Harvey,[94] and Goodsell and Dickerson,[95] with somewhat conflicting results. Liu and Beveridge[27] recently revisited the problem of fitting the data to a dinucleotide model using Monte Carlo simulated annealing and claimed to reconcile the gel retardation data with crystal structure data. This study indicated that the ApA wedge from regression analysis was not a unique solution to the problem and, if the uncertainty in the parameters derived from crystallography is considered, the crystal structures and R_L data base are not necessarily in

conflict. Also, the Liu et al. (LB) model was able to fit an essentially straight A-tract model to the data and succeeded in accounting for the relative curvature of the set of unconventional helix phasing sequences of Dlakic and Harrington,[21] previously thought to require at least a trinucleotide model. Hardwidge and Maher[105] tested various models and found Liu et al. to have the best predictive power to date on ligand ladder experiments with subtle helix phasings. Kanhere and Bansal have substantially extended the range of studies in this vein and, based on their CS model,[68,106,107] which performed well and requires no fitting, also concluded that gel retardation results are not in conflict with crystal structure data. Bansal and coworkers found that trinucleotide models to date proved relatively unsuccessful in accounting for the experimental data on axis curvature. From the collective studies, we have learned that more than one model at the dinucleotide level fits the data, and that the experimental data base is not robust enough to resolve the distinctions unequivocally. Furthermore, the nature of DNA curvature cannot ultimately be unequivocally established at this level of theory, as all of the studies of this genre are just ways of obtaining what was referred to above as a knowledge based structural construct, not a physical all-atom DNA structure or ab initio model.

Monte Carlo Metropolis simulations applied to DNA curvature problem produced early useful theoretical insights into the nature of DNA curvature and flexibility referenced to biophysical measures. Ulyanov and Zhurkin[17] used DNA energy functions to study the sequence-dependent anisotropic curvature and flexibility of DNA; even with the neglect of explicit solvent, the idea that local deformations (kinks) toward the major groove at YpR steps emerged from this analysis. Subsequently, Zhurkin et al.[24] applied Monte Carlo simulation on DNA to the problem, elaborated the idea, noted the possibility of context effects, and made the distinction between static and statistical bending, and issue of curvature vs. flexibility. Subsequent Monte Carlo simulations were reported by Maroun and Olson[99] (in the context of polymer statistics), and by Shore and Baldwin[108,109] and Jernigan and coworkers[25,110], all of which are discussed in more detail by Olson and Zhurkin.[26] A promising alternative approach to the study of DNA curvature and bending based on MD simulations is being pursued independently by Lankas et al.[111] The vehicle for this analysis is the sequence-dependent elastic rod model of DNA developed in terms of parameters that can be identified with a Young's modulus tensor with respect to any well-defined subunit and define a flexible anisotropic rod model[112] for the DNA representative of the MD results. Details of the MD procedure to obtain the elements of the elasticity temperature have been described by Lankas et al.[111] with reference to pentameric tracts; further studies in this vein are currently being carried out by F. Lankas, J. H. Maddocks and colleagues (private communication).

The observed enhancement of DNA curvature by the presence of phased A-tracts makes it especially important to understand the dinucleotide ApA step as fully as possible. A:T base pairs exhibit a higher intra-base pair propeller deformation than C:G, a simple consequence of two Watson–Crick hydrogen bonds between base pairs, rather than three. Experimental data based on crystal structures[113] and theoretical energy calculations[17,24,110,114] indicate that ApA steps show little tendency for deformation or deformability with respect to B-DNA. An explanation has been offered in terms of steric clashes due to the large propeller ("a stack of carpenter's sawhorses is more difficult to push over than a stack of flat wooden planks"[115]) and particularly favorable base pair stacking.[110,115,116] Hunter's analysis[114] suggests that a steric clash between the T-CH_3 group in the major groove and the neighboring 5′ sugar ring is at least partly responsible. Crystallography[117] and resonance Raman spectroscopy[118,119] suggest that an additional "bifurcated" H-bond between cross-strand A-N6 and T-O4 may be a source of the stability. Whatever the reason, ApA steps in crystal structures of uncomplexed DNA turn out to be essentially straight and relatively rigid[113] and support the non-A-tract model as opposed to the ApA wedge model. Contributing to the dispute over the relevance of DNA crystal structures to solution phenomena was the recent report that the rigidity of ApA steps in crystals may be sensitive to the effects of organic solvents such as MPD, a common co-crystallizing agent in the preparation of samples for X-ray diffraction studies.[53] However, the nature of this effect is disputed,[115] and the results can be explained based on any of the proposed ad hoc models of DNA curvature.[120]

Finally, even if essentially straight, A-tracts are expected to deviate slightly from canonical B-form DNA structure and adopt the B′ form (Figure 1c), characterized by high *propeller*, negative base pair *inclination*, and a progressive narrowing of the minor groove 5′–3′ within the A-tract. The B′ form of DNA is seen clearly both in fiber diffraction structures of the homopolymer poly d(A:T)[121] as well as in crystal structures of oligomeric A-tract sequences[113] and invoked in the interpretation of hydroxyl radical footprinting experiments.[122] Also, ApA steps in crystals are not perfectly straight, but they exhibit on the

average small nonzero values of *roll* and *tilt*, −0.89 (±2.87) and −0.11 (±2.50).[123] This results in a gentle "3D-writhe" of DNA double helix about a hypothetically straight reference axis, which has been observed to be a general property of all canonical forms of DNA.[115] A comparison of 3D-writhe for A, B, and B′ DNA is shown in Figure 1. As is evident from Figure 1c, 3-D writhe is particularly small in A-tracts compared with canonical A- and B-form DNA (Figure 1a and 1b, respectively) and leads to significantly less macroscopic curvature than sequence elements with high deformation and deformation elements such as YpR. In particular, 3D-writhe in B-DNA is not to be confused with an ApA "wedge," which is typically associated with an order of magnitude higher values of *roll.*[17,91]

DNA structure has been known from the earliest of studies by Franklin and Gosling[35] to be sensitive to solvent effects, with B-form preferentially stabilized under conditions of high water activity, and B-to-A transition induced by lowering the humidity of a fiber of the water activity of A-philic sequences in solution. Thus, the effects of water molecules and mobile ions in solution may well play a significant role in sequence dependent DNA curvature and flexibility. The minor groove of B-form DNA in crystals features a characteristic "spine of hydration" that stabilizes the structure,[124] although the vice-versa cannot be so easily dismissed in the absence of a causal connection with free energy.[125] Recently, evidence from MD simulation,[10] crystallography,[126] and NMR spectroscopy[127,128] has been provided in support of the idea of fractional occupation of counterions in the grooves of DNA. This introduces another possible source of sequence effects, and may contribute to the stability of A tracts,[10,127–129] and possibly G-tracts as well.[16,130] Recent crystal structures in the presence of heavier counterions show increased fractional occupancies,[13,131–133] as much as 50% in the case of $T1^{+}$.[134] There is leading evidence as well as a proposed model for ions in the grooves of DNA as bending loci[12,135,136] with the structures referred to as "bending polarons" in the studies of divalent cations around DNA by Rouzina and Bloomfield.[12] In this type of mechanism, the self-localization of cations at the major groove entrance is accompanied by collapse of the groove and DNA bending and is driven by nonbonded electrostatic attraction between the compact cationic charge and the anionic phosphates from both strands of the incipiently collapsed groove.[13] This idea has been generalized as the "flexible ionophore" model of DNA curvature,[14] in which A-tracts are sites of significant ion occupancy in the minor groove and G-tracts are sites of significant ion occupancy in the major groove, where the G-O6 atoms form an electronegative pocket. Just what stabilizes what in terms of free energy is a "chicken and egg" problem that remains to be unequivocally clarified. The net effect is that A-tracts assume a B′-form and induce a deformation toward the minor groove, while G-tracts preferentially move locally in the direction of A-DNA and induce curvature toward the major groove. The net DNA curvature is the result of a "tug of war" between these two tendencies when both are present.[16] To our reading, this may be a direct effect on curvature, or else a factor contributing to why A-tracts and G-tracts are differentiated in structure from normal B-DNA. In the latter case, "R-tracts" establish a phasing frame, and thus serve to enhance bending that actually originates elsewhere in the sequence.

In summary, the various ideas about the structural origins of axis curvature in phased A-tract sequences differ with respect to whether A-tracts in solution are straight or bent, exactly where the main origin of bending lies in the sequence, and the role of intrinsic versus environmental effects in sequence-dependent structural preferences of DNA. In the ApA wedge model, the main origin of intrinsic curvature lies within the A-tracts per se. In the case of straight A tract models, axis deformations could occur at the junctions of the A-tracts (B′-form) and normal DNA (B-form). The main origin of curvature in the junction model is at the articulation of B′-form and B-form sequence elements, with the curvature likewise amplified by helix phasing. In the non-A-tract, YpR kink model, the origin of deformation occurs not just at junctions, but at various locations in the non-A-tract regions of sequence. The structural deformation "at or near" an interface of structurally differentiated sequence elements in junction models may in some cases be a special case, as indicated by the DNA crystal structures.[8] Whether local fluctuations in ion occupancies are a causative factor, or a consequence, of sequence-dependent DNA curvature remains to be fully established.

METHODS

MD simulation is a computer "experiment" in which the atoms of a postulated system execute Newtonian dynamics on an assumed potential energy surface. The MD procedures specific for biological macromolecules are described in detail in the recent monographs of McCammon and Harvey,[137] Leach,[138] and Schlick.[139] The model system chosen for study, the assumed potential energy surface

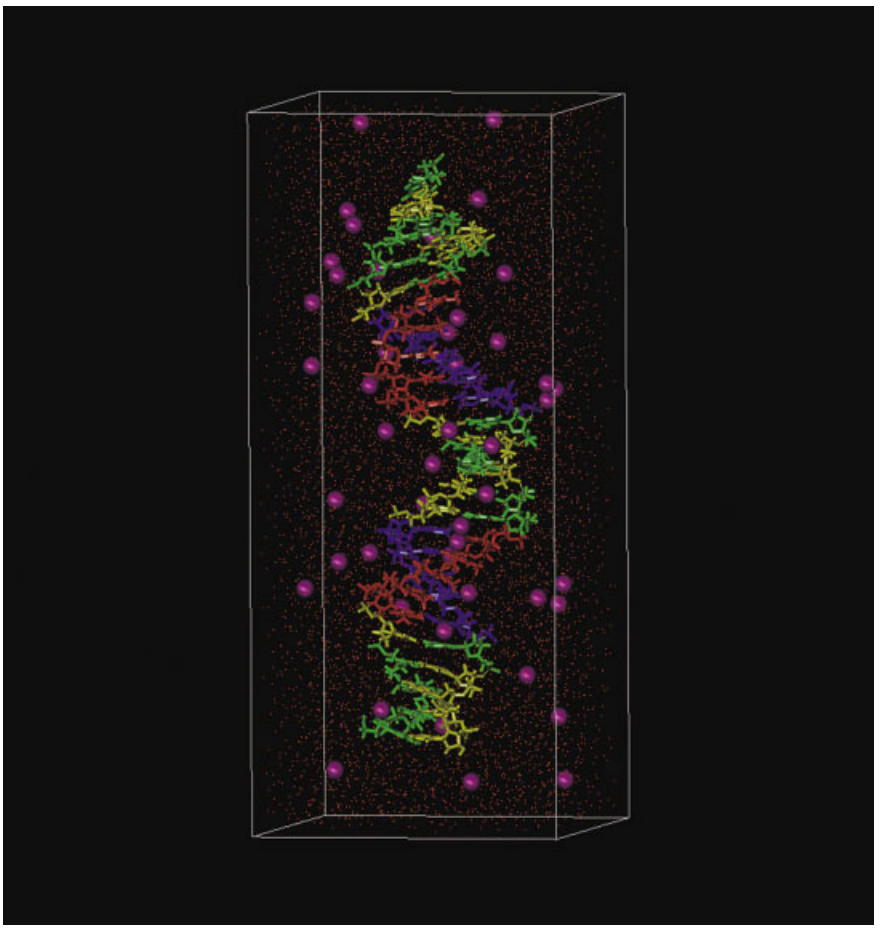

FIGURE 3 DNA, water and ions in a typical simulation cell used in the simulations described in this article.

(force field), and the simulation protocol are all operational variables in the calculation. Unless otherwise specified, all calculations employed the all-atom AMBER parm94 force field developed by Cornell et al.[140] and the TIP3P model for water,[141] and were performed using the AMBER suite of programs.[142] The initial configuration of the system is first subjected to energy minimization to relieve any major stresses, followed by period in which the particle velocities are increased to correspond with the temperature of interest (heating). The MD proceeds via Newtonian dynamics to locate a thermally bounded state (equilibration) and to sample it (production). Analysis of the results proceeds from the ensemble of individual structures (snapshots) which comprise the production segment of the simulation. Assuming ergodicity, this ensemble approximates to a Boltzmann distribution after sufficiently long period of sampling.

A typical MD simulation on a DNA oligonucleotide begins with the choice of an initial configuration and an arbitrary arrangement of solvent water and counterions in a simulation cell of appropriate dimensions. An example from one of our most recent simulations is shown in Figure 3. Sufficient water is included to provide a solvation shell of $\geq$10-Å thickness, which surrounds the DNA with $\sim$10,000 solvent molecules depending on the length of the sequence. The calculations assume periodic boundary conditions, i.e., a representation of the aqueous solution as a quasi-crystal of liquid density. However, with sufficient solvent included and the DNA located at the center of the cell, the environment of the DNA approximates well to aqueous solution.[143] Sufficient ions are added to provide electroneutrality (minimal salt condition), and further ions and coions are added to achieve a given environmental composition and ionic strength. The details of individual simulations in this survey differ slightly (see below). Earlier studies in this survey placed the DNA molecule at the center of a rectangular prism or hexagonal prism. The simulation was performed with respect to the center of mass of the DNA properly oriented in the box by continuously removing translations and rotations. Most recent MD on DNA uses a truncated octahedral cell in which these adjustments are no longer necessary. We have found the results on DNA bending to be unaffected by this transformation.

In a typical MD protocol, heating and equilibration steps are carried out as gradually as possible, to maintain the conditions on the equations of motion with a proper radius of convergence. Heating to the desired temperature involves

~100 ps, with harmonic constraints initially restricting the motion of the DNA. These constraints are slowly relaxed under constant temperature and volume conditions, each run being supplemented by ~1,000 steps of energy minimization to make sure any clashes are relieved. The simulations were then continued, using constant temperature (300 K) and pressure (1 bar) conditions, using the Berendsen algorithm.[144] Long-range interactions are treated with PME, typically using a with a real space cutoff of 8 Å and a grid spacing of 1 Å. SHAKE constraints[145] were applied to all bonds involving hydrogen atoms. The MD integration time step was 2 fs. Equilibration and production steps of the calculation were extended as noted above during which conformations of the system were saved every 0.5 ps for further analysis. The MD simulations discussed in this review involve from 5 to 50 ns of production time. Conformational and helicoidal parameters were calculated using the program *CURVES*[146] with the local parameters option, using the version currently implemented in the Molecular Dynamics Tool Chest (MDTC).[147]

Analysis of DNA from an MD model requires some special consideration, since there is no well-defined expectation value for this property. Thus, there are several alternative ways to proceed. It is best to look at this in two parts; determination of the magnitude of axis bending and the direction. The experimental data from biophysical studies are typically reduced to a single generalized angle of curvature α. Interpreting α in terms of molecular structure requires assuming that DNA curvature is monotonic and unidirectional, and implicit in this interpretation is the idea of a rigid structural construct. From an all-atom structure point of view, one must consider the general situation in which the net overall deformation of a sequence may be the resultant of various local deformations. A given value of α may arise in different ways even at the level of intrinsic curvature, and is not uniquely defined. With a calculated dynamical model from MD, for any given structure, some report the angle between the molecular planes of the first and last base pairs in the sequence, assuming they are not artifacts of end effects. A global axis for a structure may be determined by spline fit as in *CURVES,* and net bending calculated as the ratio of the end-to-end distance and the helix path length. However, this could fail to distinguish between curvature and contraction, if the latter is a problem. Another approach is that of *MADBEND.*[103] The best approach is generally to calculate all possible measures, and compare. The curvature and flexibility step by step in a sequence can be examined by polar plots of *roll* and *tilt* angles called "bending dials," of which there are at least two variants.[8,103] In fact, a number of such maps are potentially of interest in this problem, such as *roll/slide.* More detailed consideration of the direction as well as magnitude of DNA curvature from MD results is central to the "persistence analysis" method proposed by Prevost et al.[148]

In order to locate precisely the origin of bending in an oligonucleotide structure, a conjoint consideration of both the overall axis curvature and local stepwise deformations is required. Our current method of choice for this task, also used by MacDonald and Lu,[45] involves an adaptation of "normal vector plots" introduced by Dickerson[149] for the analysis of helix bending in protein DNA complexes. As illustrated in Figure 4, normal vector plots involve raising a unit vector perpendicular to each base pair in the sequence (Figure 4a), bringing them all to a common origin (Figure 4b) and viewing the results from the axial direction, or "birds-eye" view (Figure 4c) with the tips of each normal vector connected in sequentially. A clustering of local bending vectors indicates a straight part of a helix, and two straight regions linked by one or more kinks are clearly differentiated as two distinct sets of such clusters linked by one or more connecting vectors. The canonical B- and A-form helices have quite characteristic presentations in normal vector plots (Figure 1) with the uniform contribution of 3D-writhe noted above clearly distinguishable from other sequence-dependent effects. To adapt Dickerson's normal vector plots to MD analysis, we generate of local bending vectors for various snapshots of the DNA along the course of an MD trajectory. The results presented in the following section include a detailed example of the application of normal vector plots to analysis of curvature in MD models and dynamical structure in general.

CALCULATIONS AND RESULTS

The topics considered in this section are (1) the accuracy of the MD models in describing the structure of DNA oligonucleotides in solution, and base pair sequence effects in general; (2) MD models of the dynamical structure of ApA steps and A-tracts; (3) the MD description of A-tract induced curvature, helix phasing, and 5′-3′ sequence polarity; and (4) the correspondence of the MD results with previously proposed ideas about of DNA curvature and flexibility.

MD Models of DNA Structure in Solution

The evolution of MD simulation as applied to DNA and related systems is available in several previous reviews from this laboratory.[150–152] Status reports on nucleic acid force fields were recently provided by Cheatham and Young[153] and MacKerell et al.[154] This laboratory has also contributed reviews on the special topics of DNA hydration,[150] the ion atmosphere of DNA,[155] and protein–DNA interactions.[156] Notable independent reviews of MD on DNA are due to Cheatham and Kollman,[157] Giudice and Lavery,[158] Norberg and Nilsson,[159] and Orozco et al.[160] In this laboratory, extensive MD simulations on the oligonucleotide d(CGCGAATTCGCG) as a prototype case have been carried out. These have been described in a series of papers reporting MD trajectories ranging

now from 5 to 60 ns (increasing as computers became more powerful), all based on *AMBER* and the parm94 force field of Cornell et al.[140] The results of MD on a crystal of four independent *Eco*RI dodecamers in a $P2_12_12_1$ unit cell have been tested directly against corresponding experimental data from crystallography[152,161] and found to agree within 1 Å root-mean-square deviation (RMSD) overall. Because of the sensitivity of results on the assumed density and environmental composition of the crystal, this turns out not to be a highly critical test of a force field. For the solution state, our initial MD results on the *Eco*RI sequence were compared with the distributions of helicoidal parameters obtained on B- and A-form DNA oligonucleotides in the crystallographic data bank.[11] The *AMBER* parm94 model of d(CGCGAATTCGCG) was confirmed to be well within the B-form DNA family of structures, and well differentiated from A-form structures. The structure of the MD calculated ion atmosphere provided independent support of the model assumed in counterion condensation theory.[11,162] The calculated hydration, when examined in detail, was robust and showed correct dielectric behavior.[163] Analysis of this MD also yielded the idea of possible intrusion of counterions into the minor groove noted above.[10] In a follow-up study, Arthanari et al.[143] compared MD results on d(CGCGAATTCGCG) in solution at in vivo ionic strength with both 2D NOESY spectra and RDC structures from NMR spectroscopy, and particularly close accord was established.

MD calculated sequence effects for the 10 unique base pair steps in DNA oligonucleotides form a basis for a more detailed comparison of theoretical and observed values, and provide a higher-resolution characterization of force fields. As noted above, the YpR, RpR (=YpY), and RpY steps over all B-form DNA crystal structures are found to fall into fairly distinct clusters. Results from MD simulations on DNA sequences based on AMBER and the parm.94 force field have been compared with the experimental data.[120,152] The MD results follow the crystallographic trends quite closely with ApA steps essentially straight, YpR steps strongly favoring deformation toward the major groove, and RpY steps showing a preference for deformations towards to minor groove, but less emphatically. From the MD results,

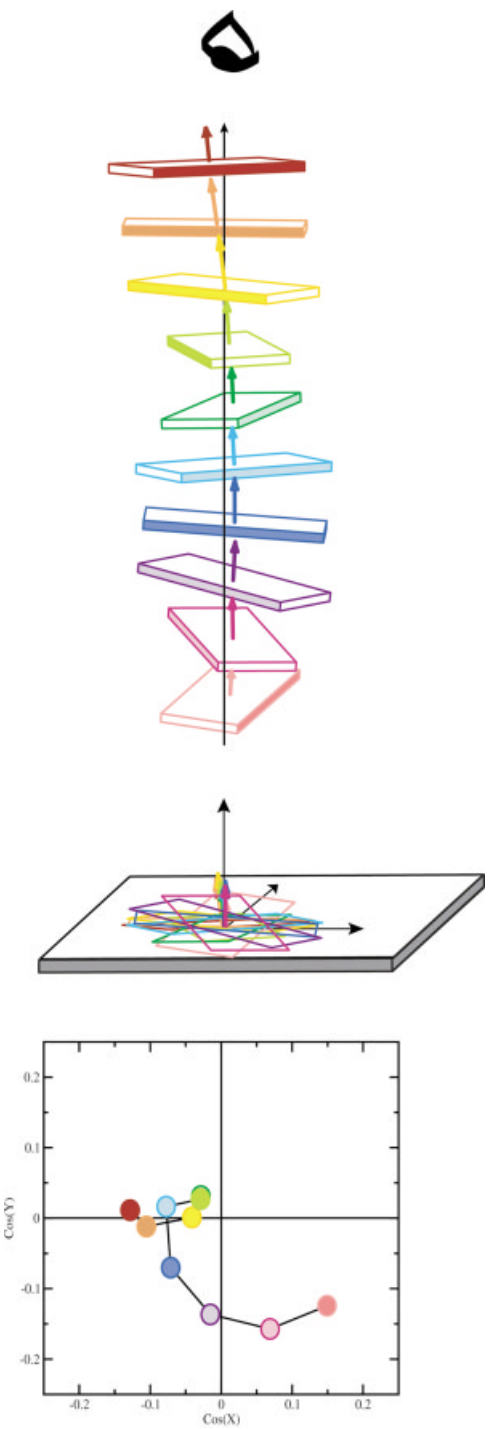

FIGURE 4 Schematic representation of a normal vector plot (NVP). The NVP is based on a view of DNA down the helical axis. Unit normal vectors are drawn perpendicular each base pair. All these normal vectors are translated to a common origin, thereby maintaining any deviation from the central helical axis. The direction cosines with respect to the x- and y-axes are plotted. Sections of DNA having a straight structure appear as clusters in the NVP, and major deviations from this helical axis appear as deviations from clusters. Keeping in mind the end effects, the overall curvature can be approximated by the vector drawn between the first and last base pairs of the sequence.

curvature and flexibility were calculated by step.[152] YpR steps were predicted to be most intrinsically curved and also most flexible, i.e., susceptible to curvature and protein-induced bending. RpR and RpY steps showed less intrinsic deformation and deformability, but trends were consistent with corresponding surveys of the crystallographic database.[63] The MD calculated flexibility by step correlated well with the bendability deduced independently from the protein–DNA crystal structures.[19] The MD results indicated that curvature and flexibility of base pair steps in uncomplexed DNA oligonucleotides are highly correlated, i.e., steps, which exhibit the most intrinsic deformation with respect to B-form DNA, turn out to be also the most deformable. Collectively, these results provide a basis on which applications of MD to studies of DNA curvature and flexibility are credible. However, the issue of stability and convergence in MD on DNA is a matter of constant concern, particularly with respect to mobile counterions (see Discussion).

MD Studies of ApA Steps and A-Tracts

The results of MD simulations on oligonucleotide ApA steps and A-tracts have been described in a series of recent papers. MD simulations including water and counterions have been reported on all examples of A-tract DNA oligonucleotide dodecamers for which crystal structures are available, the homopolymeric sequences poly dA and poly dG, and two related sequences that serve as controls.[120] MD describes the distinctive B′-structure of A-tracts in solution as essentially straight (wedge angles of $<1°$), more rigid than generic B-form DNA, with the high propeller twist, slight negative base pair inclination, and 5′ to 3′ minor groove narrowing of canonical B′ form quite well reproduced. The MD structures of ApA steps and A-tracts in solution agree closely with corresponding crystal structures, supporting the idea that crystal structures provide a good model for A-tract DNA structure in solution. Furthermore, it is clear that minor grove narrowing can be achieved via cooperative effects in straight A-tracts and that it does not require A-tracts to be curved per se.

The origin of the characteristic structure and relative rigidity of ApA steps and A-tracts as discussed above has been attributed to a combination of steric and electrostatic effects, possibly supplemented with a cross-strand bifurcated hydrogen bond. In further detailed studies, MD was used to compare the structure and dynamics of the sequences AAA and the inosine (I) mutant AIA, in which there is structurally no opportunity for a bifurcated hydrogen bond to form. The results indicate that the inosine substitution does more to the dynamical structure of the oligonucleotides than might be expected from just eliminating a bifurcated hydrogen bond across the major groove, and point to the importance of DNA flexibility as much as static structure in determining macroscopic behavior. It was suggested that the foreshortened N6-H$\cdots$ TO4 distance associated with the bifurcated H-bond across the major groove could arise as a de facto consequence of characteristic high propeller twist in A-tracts, rather than as the driving force behind it. Earlier MD on A-tract oligonucleotides,[164] as well as more recent calculations,[165,166] indicate that structures with possible bifurcated hydrogen bonding comprised only a small fraction of the trajectory, while other features of A-tracts, such as minor groove narrowing, remain intact. Examination of AAA steps in 17 different oligonucleotide crystal structures reveals that only 13% of the examples show structures with interatomic N6-H$\cdots$ O4 distances of $\leq$2.8 Å.[167] However, NMR[168] and resonance Raman studies,[118,119] explicitly support an interaction, and the issue may reduce to how much free energy of stabilization is contributed by this interaction. The net contribution from each instance would be expected to be small at best, but could, of course, be cumulative. Pastor et al.[166] have visited this issue with simulations on the TATA box sequence d(CTATAAAAGGGC) and a similar sequence substituting the A with I. These investigators find as well that I-substitution introduces more flexibility, and independently conclude that the bifurcated H-bond in the major groove does not contribute to the stability of the A-tract over the I-tract.

MD Studies of Phased A-Tracts

Focusing on one of the essential features of the DNA curvature, an MD study of a DNA oligonucleotide duplex featuring A-tracks phased by a full helix turn was first reported by Young and Beveridge.[169] Specifically, a series of nanosecond-level MD simulations were performed on a 25-base pair phased A-track duplex of sequence d(ATAGGCAAAAAATAGGCAAAAATGG), phased at 11 bp pairs per turn, for various saline compositions. A 30-bp duplex composed of three 10-bp repeats of the *Bam*HI recognition sequence was simulated as a control. The MD results, for a concentration of 60 mM KCl, 10 mM $MgCl_2$ added salt plus minimal neutralizing cations, exhibited concerted axis bending to the extent of $\sim$16.5° per A-tract. This compared favorably with the bending per turn of 17–21° inferred from experiments.[83,84] The A-tracts in the MD model were found to be

essentially straight. The origin of axis curvature in the MD model was located at the three YpR base pair steps in the sequence TpA, CpG, and CpA. A subsequent MD study by Sprous et al.[170] was aimed at investigating aspects of sequence polarity in DNA structure and curvature at the level of dynamical structure. MD was performed on the DNA duplexes $d(G_5\text{-}\{GA_4T_4C\}_2\text{-}C_5)$ and $d(G_5\text{-}\{GT_4A_4C\}_2\text{-}C_5)$ to 3.0 ns and 2.5 ns, respectively, at ionic strengths comparable to that of a ligase buffer. Analysis of the results showed that the $d(G_5\text{-}\{GA_4T_4C\}_2\text{-}C_5)$ simulation to exhibit strong gross curvature, consistent with experiment.[85] The primary locus of curvature in the MD structure for the phased GA_4T_4C motif was found at the central C15—G16, a YpR step, with an average roll angle of 12.8° ± 6.40°, with the A-tracts on average essentially straight. The dynamical structure of $d(G_5\text{-}\{GA_4T_4C\}_2\text{-}C_5)$ exhibited minor groove deformation comprised of expansion at the 5′ end and progressive narrowing toward the 3′ end, supporting the interpretation of hydroxyl radical footprinting results.[171,172] For the corresponding T_4A_4 motif, the TpA steps are out of phase with the other CpG bending elements, resulting in a net compensation effect and reduced overall axis bending in the sequence. In summary, in this initial round of studies on phased A-tracts, MD simulation was found to recover a number of the essential features of DNA curvature previously revealed by experiments, and to provide a description of DNA curvature substantially in accord with the non-A-tract, YpR kink model. In retrospect, each of these simulations is short with respect to our present standards as discussed below, and the situation with respect to added salt is not fully resolved because of the particularly slow convergence of the mobile counterions.[153] An update on convergence issues with respect environmental counterions and coions is provided below.

Our next project was aimed at benchmarking the extent of curvature in a phased A-tract sequence at minimal salt concentration including only sufficient Na^+ cations to provide electroneutrality to the system, and to carry this over to a study of temperature dependence. The sequence chosen for this study was the duplex DNA d(GGCCGAAAAACCGCGAAAAACGGCG), exhibiting A-tracts phased at 10 base pairs per turn, and the subject of an extensive calorimetric investigation of the premelting transition and its relevance to the problem of DNA curvature by Breslauer and coworkers.[9,173] Average structures over 3 ns of MD trajectory at 303 K are shown in Figure 5. The minimal salt MD in this case (Figure 5a) showed clearly concerted overall curvature compared with the average structure at 313 K, Figure 5b. The nature of

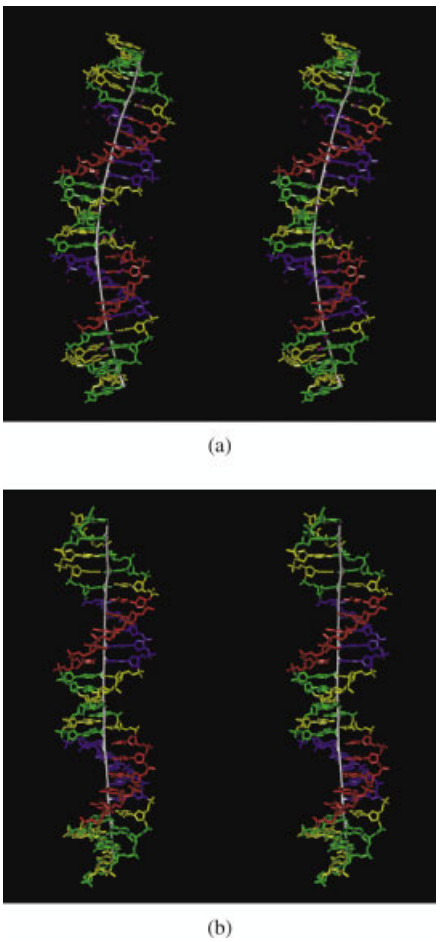

FIGURE 5 Stereo view of the average structures of A-tract containing DNA sequences obtained from 3-ns molecular dynamics simulation of d(GGCCGAAAAACCGCGAAAAACGGCG) for (a) 303 K and (b) 313K. The adenine and thymine bases in the DNA are shown in blue and red, respectively, while the guanine and cytosine bases are shown in green and yellow.

the curvature based on normal vector analysis of snapshots from the MD trajectory is shown in Figure 6, which provides a dynamical view of the axis curvature from MD. The average MD structure was computed and the analysis of this construct, is shown in Figure 7. The tips of the vectors normal to the A:T base pairs within both A-tracts are well aligned, indicative of essentially straight helices. The diameter of the semicircle formed by the tips of the normal vectors of bases within the A-tracts is slightly smaller than that of B-DNA indicative of the smaller 3D-writhe typical of B′-form DNA. In contrast, the normal vector tips for base pairs in the non-A-tract region of the sequence are in some cases widely separated, particularly across the internal CpG steps in the spacer elements as well as at the 3′ terminus. An analysis

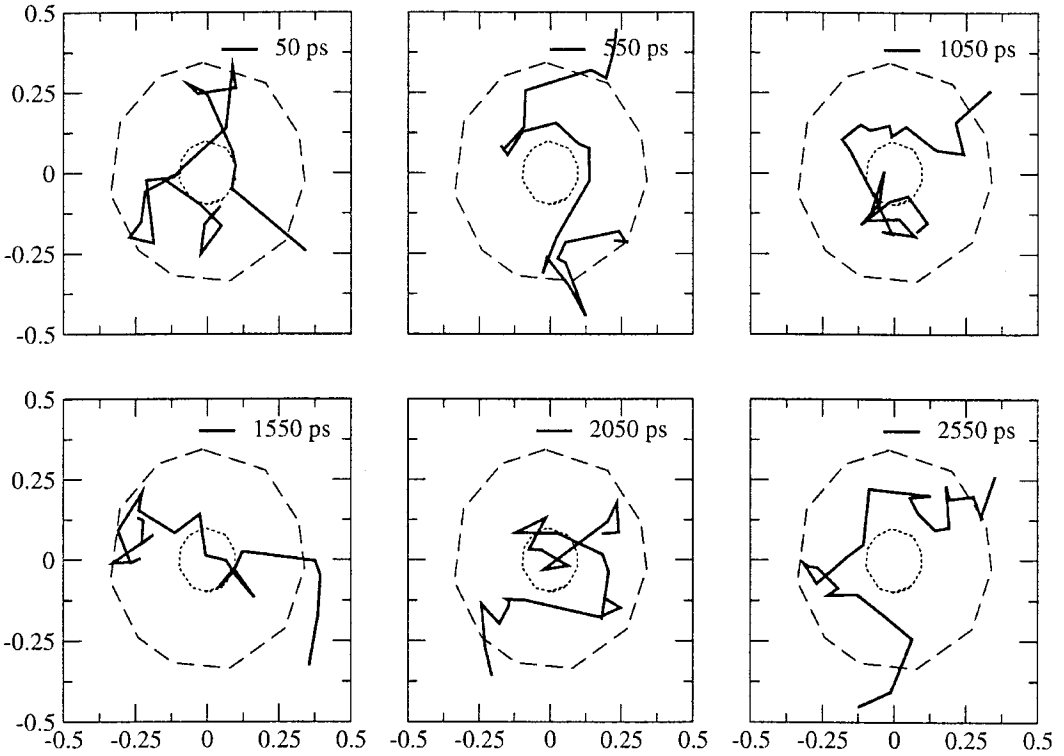

FIGURE 6 Normal vector plot analysis of the curvature of d(GGCCGAAAAACCGC-GAAAAACGGCG) during the simulation of the molecule at 303 K. The concentric rings in the center of the plot show the curvature due to 3D-writhe in A (dotted curve) and B (dashed curve) form DNA, respectively.

quantitative resolution of the various contributions by step to the net overall bending calculated from the normal vector plot analysis of the MD on this sequence is also shown in Figure 7. Three of the five CpG steps account for more than two-thirds of the total, and thus comprises the primary origin of curvature in the MD model. However not all CpG steps in the sequence contribute, consistent with the idea that there are thermally accessible substates of YpR sequence elements and that this step may behave as a flexible hinge. Examination of *roll/slide* maps for all CpG steps of this sequence indicates that the curved YpR elements reside in the open hinge (high *roll*/ locally A) substate, while the other two reside in the closed hinge (low *roll*/locally B-form) substate.

Experimental observations linking DNA curvature with phased A-tracts are, however, unequivocal and indisputable. Thus, our MD result that the origin of bending in this sequence lies in the CpG steps rather that the A-tracts raises the question, what role do the phased A-tracts play in DNA curvature? What is the true nature of the helix-phasing phenomena at the molecular level? This question is best pursued by focusing on the subset of normal vectors in Figure 7 for the interior . . .AAAAACCGCGAAAAA.. sequence element, selected so as to be free from end effects. The distinct clustering of vector tips for the A-tracts analysis dictates that the-A tracts be considered together as a relatively rigid structural subunit of the sequence. The net effect of the combination of YpR hinges and phased A-tracts appears to be that the vector connecting the 3′ end of one A-tract with the 5′ end of the other A-tract effectively orients the two A-tract structural units parallel to that of the direction of overall curvature for the sequence (the vector connecting the tips of the terminal 5′ and 3′ bases). When this condition is met, the A-tracts are each disposed along the circumference of a single great circle, leading by construction to a state of maximum concerted curvature. Furthermore, the rigidity of the A-tracts as structural elements contributes to the precision with which this constructive effect is accomplished. A more flexible structural element in this position would reduce the precision with which the elements combine, leading to less effective concerted bending. Thus the phasing of as many relatively rigid structural elements as possible is the key to maximal curvature, and the particular structural rigidity of A-tracts intro-

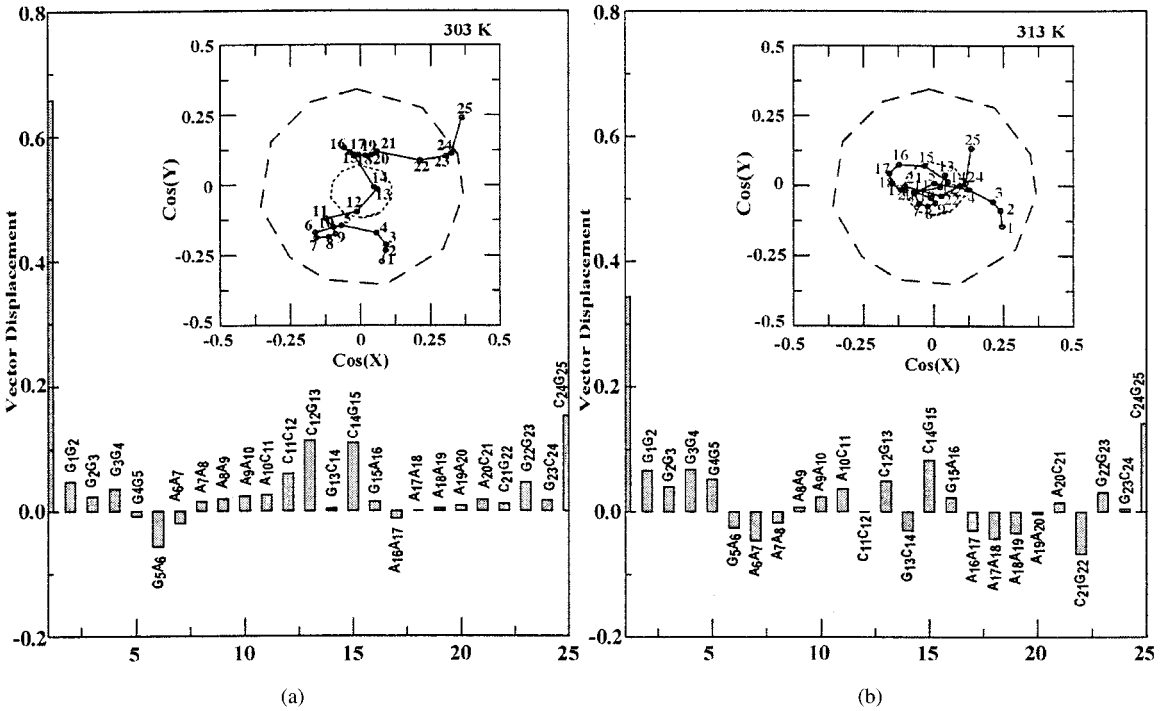

FIGURE 7 Normal vector plot (inset) and vector resolution of the individual contributions of the curvature arising at each of the steps in the A-tract containing DNA molecule simulated at 303 K (left) and 313 K (right). Inset in each of the images is the normal vector plot used to calculate the curvature between consecutive steps in the MD average structure DNA.

duces precision into the way the YpR elements act cooperatively to produce enhanced curvature.

MD Studies of the B′-to-B Transition

Fiber diffraction, crystal structures, spectroscopy, and footprinting experiments indicate that A-tracts in DNA oligonucleotides assume the B′ form of right-handed DNA, with a narrow minor groove, and high propeller and negative inclination. As noted above, the B′ form structure of A-tracts has previously been shown to be well described and accounted for by MD simulation.[120] Calorimetry[9,173,174] and UVRR spectroscopy[118] studies of B′ forms of DNA as a function of temperature has revealed the presence of a so-called premelting transition, associated with the conversion of the B′- form in the A-tracts to regular B-form DNA. Above the premelting transition, axis curvature is reduced.[173] Does MD provide an account of this phenomenon? To answer this, MD on the 25-mer sequence d(GGCCGAAAAACCGCGAAA-AACGGCG) in solution at eight different temperatures between 273 and 338 K was carried out and analyzed using normal vector plots in the manner described above. The results are as follows: the net axis curvature of the DNA increases regularly up to ~300°–310° consistent with the increasing thermal population of higher energy states of an anisotropic potential surface. In the region between 310 K and 330 K, a discontinuity is observed in the temperature dependence of selected MD indices of structure and, as indicated in the normal vector plots of Figure 7, the overall curvature of the DNA sequence decreases, a result consistent with several experimental reports.[20,175,176] This occurs very near the observed B′-to-B premelting transition of 228–330 K,[118,173] indicating that MD succeeds in providing a proper theoretical account of the premelting transition.

The molecular mechanism of the premelting transition provided by MD turns out to be more complicated than simply a B′-to-B transition in the A-tracts. The normal vector plots for the MD of the 25-mer sequence on either side of the pre-melting transition are shown in the two panels of Figure 7. On the left side (Figure 7a), the structural characteristics of the MD in this region are similar to that of the ambient temperature form, featuring A-tracts in the B′ conformational state. For the MD carried out at a temperature just above the premelting transition (Figure 7b),

the A-tracts are shifted to B-form, and the calculated results support the idea that a B′-to-B form structural transition is involved. Corresponding results on a non-A-tract control sequence do not show this effect. Furthermore, the overall axis bending is reduced. How does this occur? There is clearly a B′-to-B transition taking place within the MD A-tracts, the net effect of which, in the manner noted above, would be reduced precision in the helix phasing or in the limiting case of no differentiation at all, elimination of phasing from A-tracts. While this alone could cause a reduction in curvature, we observe as well an MD conformational transition in YpR steps from the local A-form, high *roll* substate to the B form, low *roll* substate, with net effect of making the elements of the sequence more collinear and reducing the curvature. In summary, MD predicts that the premelting transition arises both as a consequence of the lesser phasing precision obtained from B-form compared with the more rigid B′ form helix element, and with a conformational transition in flexible YpR hinge from the open to closed state.

The decrease in axis bending with temperature observed in AT-rich DNA is thermodynamically non-intuitive, as one might expect an ensemble of straighter DNA structures to be more ordered and thus lower in entropy than ensembles of curved structures. However, closer examination of both components of the premelting transition shows that both the B′-to-B transition in A-tracts and the YpR shift from open to closed hinge states are associated with an increase in both enthalpy and entropy (Figure 8). Arguing based on a simple two-state model, the B′ form of an A-tract is both lower in energy and more rigid than the B-form, and so a B′-to-B in A-tracts occurs with an entropy increase. The open state of the YpR hinge, being a locally A-like form, is likely to be more ordered and of lower energy that the closed state, which is B-like, and thus more flexible. Thus both the B′ substate and the open hinge form of the YpR step are associated with a lower and relatively narrow potential energy well. The final state of the premelting transition is B-form in general, in which both the A-tract and the closed form of the YpR hinge lie slightly higher in energy, and show a broader potential energy well. At low temperatures, only the more rigid state is significantly populated. At higher temperature, the more flexible B-like states becomes thermally populated and, as a consequence of a higher statistical weight, dominate the equilibrium. In this argument, the premelting transition in DNA is entropy controlled.

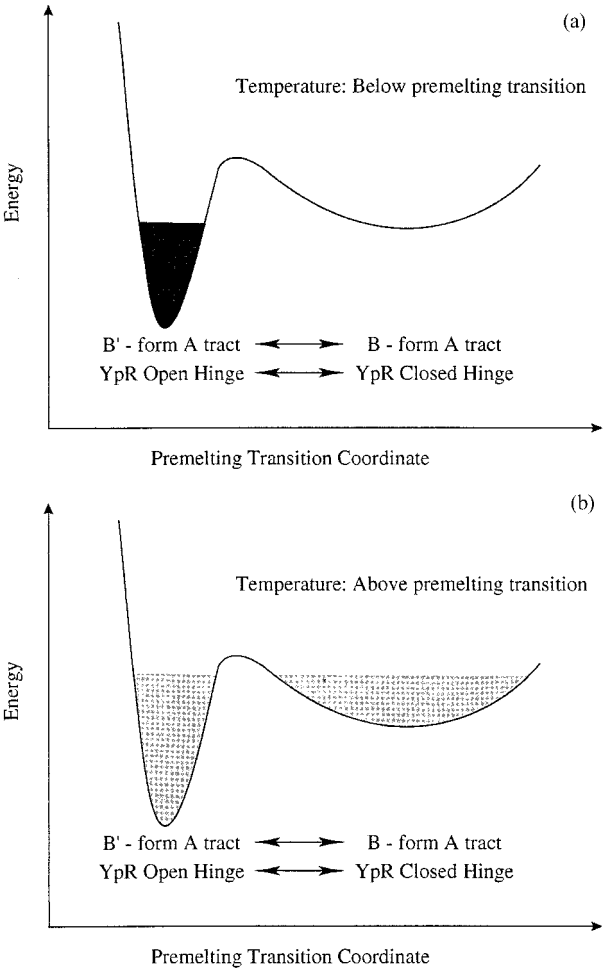

FIGURE 8 A proposed two state potential energy surface depicting the premelting transition in A tract containing DNA. The bottom and top graphs shows schematically the occupancy of states below and above the premelting transition temperature, respectively. The low energy and relatively narrow substate in the energy surface corresponds to the rigid B′ form of the A tract DNA and an open hinge/high roll state of the YpR step. This is the predominantly occupied state below the premelting transition temperature. A second higher energy substate, which is entropically favored, is occupied above the premelting transition temperature and corresponds to the more flexible B-form like structure of the A-tract and the closed hinge/low roll YpR conformation. Note that it is essentially coincidental that the same schematic diagram can be applied to potential surface for both the B to B′ and YpR hinge transitions.

DISCUSSION

The collective results on DNA curvature and flexibility reviewed in the previous section indicate that MD simulation successfully describes the phenomenon of enhanced axis curvature in phased A-tracts, even un-

der minimal salt conditions. The A-tract and the non-A-tract regions together comprise the phasing element, not just the A-tract. Below the premelting temperature, the structure of A-tracts in all MD simulations to date corresponds to essentially straight, B′-form DNA. The MD results are consistent with diverse results in the literature that suggest that YpR steps are potentially flexible hinge points. The hinge involves two substates, a locally A-form (high *roll,* deformed toward the major groove) and locally B-form (low *roll,* essentially straight). *Slide* is not as variable in the MD as in Hunter's theoretical analysis[73] and shows up slightly negative. The MD results indicate that the open hinge substate of YpR steps, when present in a sequence and thermally populated, is the motif most likely to serve as the origin of axis curvature in DNA. Such structures are subsequently referred to as *curvature elements* in the helix-phasing phenomena. In the frequently encountered case of A-tracts combined with a flanking sequence containing YpR steps, the relatively straight, rigid A-tracts are not an origin of bending per se but are integral to the phenomenon. The MD results suggest that A-tracts act as *positioning elements* that make helix phasing as precise as possible, the result of which is maximum concerted curvature. A sequence of ordinary B-form DNA is typically more flexible than A-tract (B′-form), and the idea is that this introduces sufficient imprecision (analogous to noise) into the helix-phasing phenomena as to mitigate the amplification effect. This hypothesis will be examined further in subsequent studies.

When YpR steps are found at the 5′ end of A-tracts, the curvature may follow a type of junction model that is a special case of a non-A-tract YpR kink model. While the YpR hinge, when present, is a dominant feature, an additional viable source of curvature is the deviation in trajectory of the helix axis when two sequence elements with different base pair inclination are juxtaposed so as to maintain maximal base pair stacking. The junction models are typically of this genre. The crystal structure of many A-tracts studied crystallographically contain the A_nCG motif at a B′/B junction. Crystal structures and MD independently concur that in this case the local deformation is more pronounced at the CpG step, rather than at the junction, an RpY step, indicating the dominance of YpR kinks over junction bending in the overall curvature when both elements are present. An intriguing case is that of a B′/A phasing motif, in A_5G_5. In this case, the issue is whether the origin of curvature comes at the ApG junction or from an uncompensated GpG wedge. We have an MD simulation in progress that will address this issue. However, most phasing sequences studied to date show an alternating B′/B structure in which the curvature is not localized at the junction. The structure of the interface is thus not one of plate tectonics, but of an adaptation distributed into the flanking sequence. An interesting issue is the context effect on YpR conformational preferences, i.e., do A-tracts preferentially stabilize the open hinge state?

The quest for bent A-tracts has been pursued in a number of recent studies aimed at elucidating links to DNA curvature. Two recent NMR structures of short oligos have addressed this issue. We have independently analyzed these structures using the method of normal vector plots. The deformation in structure of d(GGCAAAAAACGG) reported by MacDonald and Lu[45] clearly has the origin of curvature primarily at a YpR step. The structure of d(GGCAAAACGG) by Crothers and coworkers[46] is described as a "delocalized bend," but in our analysis 75% of the curvature lies in YpR steps and the net bending originating within the A-tract is small. Efforts to locate bent ApA steps or A-tracts in short oligonucleotides run the risk of seeing 3D-writhe, which is actually quite small in canonical B′-form DNA and not likely to be significant origin of overall curvature in a sequence. In general, to advance the case for bent A-tracts in DNA curvature, one must show ApA steps that are deformed over and above 3D-writhe and demonstrate this to be a significant contribution to the origin of overall curvature in a sequence of phased A-tracts compared with other possibilities, a point that is not yet well established. We can envisage a special case in which this is a possibility, however. In canonical forms of DNA, 3D-writhe is a compensated phenomenon, i.e., canceled out by helix phasing of ~10-bp units. As suggested early on by Calladine et al.,[98] 3D-writhe could contribute a little to curvature if uncompensated, i.e., from tracts shorter than ~10-bp units. If a sequence has no net curvature elsewhere, the small contributions from uncompensated 3D-writhe could add up. However, in most cases, DNA curvature will be dominated by the more robust elements of curvature, as discussed above.

We conclude this review with a retrospective on how well the MD results account for the leading experimental observations about DNA curvature (for references, see above). Enhanced curvature and sequence polarity effects in phased A-tracts are well described by MD. MD reports of curvature per ApA step and per A-tract are in reasonable accord with values reported experimentally, although our results indicate that the actual origin of bending is in the non-A-tract YpR steps. Reporting curvature with respect to the full repeating motif seems a better way to

proceed. By inspection, we have determined that, even though the A-tracts per se are essentially straight, the overall direction of bending in the MD structures of phased A-tracts is toward the minor groove when viewed from opposite the A-tracts, in accord with the experimental findings. However, the primary origin of curvature lies in base pair roll of the YpR steps toward the major groove, approximately half a helix turn away, which produces the same net effect on the overall curvature. The sequence polarity of curvature in A_4T_4 vs. T_4A_4 motifs is well reproduced by MD, with the lesser curvature of the latter motif a consequence of the contravening influence of TpA step out of phase with respect to other elements of curvature. Finally, the observation that DNA curvature in sequences of phased A-tracts with flanking sequences is independent of the composition, which could imply that the curvature resides within the A-tracts. However, revisiting these studies, the various flanking sequences studied always have one or more YpR steps, which according to the MD model would be the actual origin of curvature. Finally, a key argument against the non-A-tract model is that if A-tracts are straight B′ form DNA, the presumed B-form, non-A-tract region must be somewhat different in structure from canonical B DNA, for which there is little precedent. However, analysis of the MD results on the flanking sequence shows that the average MD structure is well within the variations typically seen in crystal structures of B-DNA oligonucleotides, and sequence effects on structure may introduce local fluctuations which, when amplified by helix phasing, may well serve as the origin of significant axis curvature.

One final point: our analysis shows that MD on DNA in solution supports the description of A-tracts in DNA obtained from crystallography. Nevertheless, the direction of bending in the crystal structures is demonstrably sensitive to the crystalline environment and packing effects. Since we maintain the origin of curvature lies outside the A-tracts, there is nothing contradictory about this. Also, direction of curvature is arguably more subtle than sequence effects per se, pointing to the likelihood that the trends in sequence effects from crystallography are still generally applicable to structures in solution, although close attention to possible artifacts is still recommended. A case study of this issue is the sequence d(GGCAAAAAACGG). In this case, the crystal structure[52] and the NMR solution structure[45] show similar structural features for the A-tracts, but the direction of curvature is significantly different. Three MD simulations have recently been performed here, using the crystal structure, the NMR structure, and canonical B form as starting structures. The results in each case converge to a solution structure in accord with that obtained in the NMR, a further point of validation for the performance of MD on DNA.

SUMMARY AND CONCLUSIONS

The results of MD studies to date on the DNA, albeit based on only a few cases, account well for the essential features of DNA curvature and flexibility observed experimentally and provide a model that elucidates the role of various factors involved at the molecular level. The MD model successfully integrates the ideas about helix phasing of relatively rigid structural elements such as A-tracts and the YpR flexible hinge and provides independent theoretical support for the Goodsell–Dickerson non-A-tract model of curvature in DNA containing the requisite sequence elements. In this description, the junction model is a case of YpR elements of curvature located at or near junctions. The MD results in accord with crystallography do not support the ApA wedge model or a significant contribution from curvature within A-tracts as the origin of DNA curvature. MD simulation has been used to study the nature of the premelting transition in DNA. The role of a B′-to-B structural transition is confirmed but, in addition, the mechanism involves an important shift in the conformation of YpR populations from an open to closed hinge substates figures significantly into the explanation of the premelting transition and the apparent decrease in DNA curvature with temperature.

The role of ions in DNA bending[13,177] was inspired in part by a combination of MD modeling studies,[10,135] crystal structures,[132,178,179] and NMR studies[128] and figures strongly in the bending polaron model[12] and a recent description of DNA curvature proposed by Hud and Plavec,[16] described above. The MD provides leading results, but at the same time it is clear that the motions of environmental ions are slow to converge. This was quantified in the review by Cheatham and Young,[153] in which the requisite palindromic symmetry in ion occupancy is only incipient in a 5-ns trajectory. We have now extended the original MD of Young et al.[10] to ~60 ns and examined Na^+ ion convergence as a function of time. The results indicate that the requisite symmetry is indeed slow to form, but is indicated to be ~90% complete at this level of sampling[180] and extrapolates to ~99% within 100 ns. Systems with divalent ions and excess salt systems are expected to be slower to stabilize, and therefore a definitive position of MD simulation on ions and DNA curvature remains to be fully estab-

lished. We note as well that this issue may influence all the MD on DNA curvature performed to date, but extensive high-performance computing will be required to clarify this matter. Preliminary analysis of results indicates the dynamical structure of the DNA may be well established in 5–10 ns, being sensitive more to mean field effects than the granularity of the counterion distributions.

Note added in proof: In response to suggestions from several colleagues in the field who have read critically a prepublication version of this article in manuscript form, several additional experimental results bear on the model of DNA curvature derived from our MD simulations. First is the recent paper by Bustamante and coworkers[1] that reports an apparent angle for DNA curvature per A-tract of 13.5°, somewhat lower than other experimental results.

The remaining additional citations all involve studies of chemical modifications of phased A-tract oligonucleotides which reduce the observed effective curvature. The effect of the neutralization of phosphates has been described by Strauss and Maher.[2–4] Note also the related theoretical studies by Gurlie and Zakrzewska[5] and by Kosikov et al.[6] The result that WC base pair modification in A-tracts reduce effective curvature has been reported in a series of papers by Kool and coworkers.[7] A possible interpretation of all of these results can be constructed in terms of an ApA wedge (bent A-tract) model of curvature, and also counterion interactions. However, in all instances there is some alteration in the A-tracts that could increase flexibility or otherwise alter the A-tract structures, which according to the MD model would reduce their efficacy as positioning elements in helix phasing. Thus all these results cited above can also be readily explained by the MD model of straight, rigid A-tracts with local deformations at non A-tract YpR steps. If bifurcated hydrogen bonding is a significant factor in A-tract rigidity, the base pair modifications could alter this and have the same effect, i.e. increasing flexibility, decreasing efficacy of helix phasing and reducing the curvature anomaly characteristic of unmodified phase A-tracts. Clarifying this issue will require additional experiments and simulations, and we only wish to note these results are not inconsistent with the proposed MD model of DNA curvature.

Some experimental results which we interpret specifically in favor of the MD model of DNA curvature comes from an earlier paper that we have previously overlooked by Hodges-Garcia and Hagerman,[8] which shows that C-Methylation in sequences of phased A-tracts with CG-containing flanking elements also reduces the observed curvature. The role of the CpG or CpA hinge as in the MD model could readily explain this.

It was suggested that we clarify that the MD model is situated in our opinion closest to the previously proposed "non A-tract" model of DNA curvature, but we do not claim it is identical all respects.

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