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"... the tyranny of the lattice ..."

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ABSTRACT **A systematic comparison of crystal structures of nine different B-DNA dodecamers, in three different space groups, with and without A-tracts, shows that crystal packing or lattice forces are of secondary importance for helix axis bending, minor-groove width, and propeller twist. While other local helix parameters may be influenced or even established by crystal packing, the properties just enumerated are determined primarily by base sequence. One and the same crystal packing scheme can accommodate a bend in one of two different directions, or no bend at all. A-tract regions of B-DNA are inherently straight and unbent, with base-pair inclination no different from that of general-sequence B-DNA. Where bends are observed at junctions between G·C and A·T regions, they always involve a roll about base-pair long axes in a direction that compresses the wide major groove and, hence, are 90° away from that necessary for the correctness of the junction model of A-tract bending. The G·C/A·T junction appears to be a flexible hinge, capable of adopting either a straight or a bent conformation under the local influence of weak crystal packing forces. Such forces therefore are a source of information about DNA deformability and not a curse to be deplored. But as an indication of the weakness of crystal packing forces, introduction of a single bromine atom in the major groove is sufficient to eliminate a bend, although brominated and unbrominated crystals are isomorphous.**

At a recent workshop on DNA–drug interactions at the Fundación Juan March in Madrid,‡ one member of the audience spoke of "the tyranny of the lattice," implying that most fine-structure features of a DNA crystal structure were imposed upon the helix by crystal packing forces and consequently were of little relevance to the structure of "free DNA." Since x-ray crystal structure analysis remains our richest source of detailed structural information about macromolecules, it is worth considering just how far the tyranny of the lattice extends.

An excellent case study is provided by the B-DNA dodecamers whose structures have been solved over the past dozen years. Nine key representatives are listed in Table 1 (1–12). They are instructive because they illustrate sequences with A-tracts and without, with both wide and narrow minor grooves, and in three different crystal packing environments: orthorhombic $P2_12_12_1$ and $P2_12_12$ and monoclinic $C2$. Careful comparison of these structures can help to show which aspects of helix structure are susceptible to crystal packing and which are not.

A-Tracts and Helix Bending in B-DNA

The first six dodecamers listed in Table 1 are of interest because they contain short central A-tracts: brief runs of four or six A·T base pairs without the disruptive T-A step, flanked by G·C regions. They are all isomorphous, packing in identical fashion into orthorhombic space group $P2_12_12_1$ and with essentially identical unit cell dimensions. They also share other structural characteristics. The minor groove is narrow in the central A-tracts, widening toward the G·C ends. A·T base pairs in the center have large propeller twist compared with the flatter G·C pairs. Most strikingly, each of the first five helices displays a sharp bend at one end, at the junction between G·C and A·T regions (GC/AT junction). The bend seen in the upper part of C-G-C-A-A-A-A-A-A-G-C-G (Fig. 1*a*) is entirely typical of these first five helices. The bend occurs at a GC/AT junction and is produced by rolling one base pair over the next along their long axes in a direction that compresses the major groove. Although most of the sequences in the upper part of Table 1 are self-complementary, meaning that the two ends of the helix are symmetrical in base sequence, they are not symmetrical in local helix structure. The 12°–19° bend at what will be termed the upper end of each helix in the crystal is not matched by a bend at the equivalent AT/GC junction at the lower end. Another way of displaying the bend in helix axis is via a normal-vector plot (see Fig. 3*a*). From this plot it is clear that the bend occurs mainly between base pairs 3 and 4, or at the GC/AT junction, whereas the A-tract of base pairs 4–8 is absolutely straight and unbent.

The difference in bending behavior at the two GC/AT junctions in each helix must be a consequence of different crystal packing forces, which are largely hydrogen-bonding in origin, on the two ends of the helix. The central base-pair regions of these sequences are isolated from intermolecular contacts, a circumstance that has proven useful for studying their complexes with groove-binding drug molecules. The microenvironments of the two ends are indeed different, as demonstrated by Dickerson *et al.* (13). But is it then fair simply to write off the bending as a crystal-induced artifact from which nothing can be learned? This point of view has been maintained in the past by DiGabriele and Steitz (9):

> Therefore, the bends exhibited by both of these A-tract crystal structures are due to the forces of packing in the crystal lattice and no conclusions about how adenine tracts bend DNA in solution can be drawn from them.

and echoed by Koo *et al.* (14):

> In spite of crystallographic analysis of A-tract-containing molecules, the origin of bending cannot be definitely stated at this time, since the direction of bending in the crystal does not conform to the results in solution [sic] . . . . . Furthermore, DiGabriele *et al.* showed that bending of the molecule in the crystal is determined primarily by crystal packing forces rather than by the sequence.

Such a pessimistic assessment is defensible only if one thinks of all DNA as possessing a sequence-determined rigid structure. Then if the "normal" GC/AT junction is straight, the bend at the upper junction of the dodecamers must be a crystal-induced distortion. Alternatively, if the "normal"

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Table 1. Sequences of key dodecamer structures

Sequence	Space group	Unit cell dimensions				Mol/ AU	Properties			Ref(s).
		a, Å	*b*, Å	*c*, Å	β, degrees		X	Y	Z	
Sequences containing short A-tracts (underlined)										
C-G-C-G-A-A-T-T-C-G-C-G	$P2_12_12_1$	24.87	40.39	66.20	90.0	1	*a*	+	+	1, 2
C-G-T-G-A-A-T-T-C-A-C-G	$P2_12_12_1$	24.94	40.78	66.13	90.0	1	*a*	+	+	3, 4
C-G-C-A-A-A-T-T-T-G-C-G	$P2_12_12_1$	24.87	40.90	65.64	90.0	1	*a*	+	+	5, 6
C-G-C-A-A-A-A-A-T-G-C-G	$P2_12_12_1$	24.54	40.32	65.86	90.0	1	*a*	+	+	7
C-G-C-A-A-A-A-A-A-G-C-G	$P2_12_12_1$	25.4	40.7	65.8	90.0	1	*a*	+	+	8, 9
C-G-C-G-A-A-T-T-^{5br}C-G-C-G	$P2_12_12_1$	24.20	40.09	63.95	90.0	1	–	+	+	10
C-G-C-G-A-A-A-A-A-A-C-G	$P2_12_12$	44.8	66.1	42.9	90.0	2	*a*	+	+	9
Key sequences without A-tracts										
C-G-C-A-A-G-C-T-G-G-C-G	$P2_12_12_1$	25.29	41.78	64.76	90.0	1	*b*	–	–	11
C-G-T-A-G-A-T-C-T-A-C-G	*C*2	64.83	35.36	25.35	92.24	1	–	+	+	12

Mol/AU = Dodecamer double-helical molecules per asymmetric unit of space group. Properties: X = Bend in helix axis? *a* = Bend in plane of Fig. 1. *b* = Bend in plane of Fig. 2 or at 90° to bend *a*. – = Straight helix, no bend. Y = Narrow minor groove in A-tracts? + = Yes. – = No. Z = Large propeller twist in A-tracts? + = Yes. – = No.

GC/AT junction is bent, then the lack of bending at the lower junction becomes a packing deformation. From either point of view, crystal forces have warped the double helix.

The situation changes if one regards the base sequence as not deforming the helix but making it deformable, not necessarily giving the helix a static bend but instead conferring an inherent bendability on that region of helix. Then the different microenvironments at the two ends of the dodecamer permit expression of two different states of a GC/AT junction: bent vs. straight. The GC/AT junction is a flexible hinge, capable of bending or not bending, under the influence of local forces. Typical local forces on the DNA double helix *in vivo* include interactions with other macromolecules such as control proteins or histone proteins in the nucleosome. Some sequences of DNA can bend easily around the bacterial cAMP receptor protein (CAP) or the histone core, for example, whereas other sequences such as poly(dA)·poly(dT) resist bending in nucleosome reconstitution experiments (15). Sequence-dependent variation in bendability is certainly an important factor in DNA recognition by proteins. But no one imagines that such a bend is rigid and preformed and that bent segments of DNA wander through solution, looking for proteins to fit inside their preformed loops. Hence the bending at a GC/AT junction at the upper end of each decamer and the lack of bending at an equivalent junction at the lower end provide us with useful information about the likely dynamic behavior of a GC/AT junction.

The first three sequences in Table 1 are self-complementary, so that the bent junction at the upper end and the unbent junction at the lower end are identical in sequence. This is not true for the next two dodecamers: the 5′ end of the A-tract A-A-A-A-A-T, for example, is different from the 3′ end (i.e., the 5′ end of the complementary sequence A-T-T-T-T-T). But DiGabriele *et al.* (7) found that their orthorhombic crystals of C-G-C-A-A-A-A-A-T-G-C-G contained a 50:50 mixture of dodecamers in "up" and "down" orientations. Because the

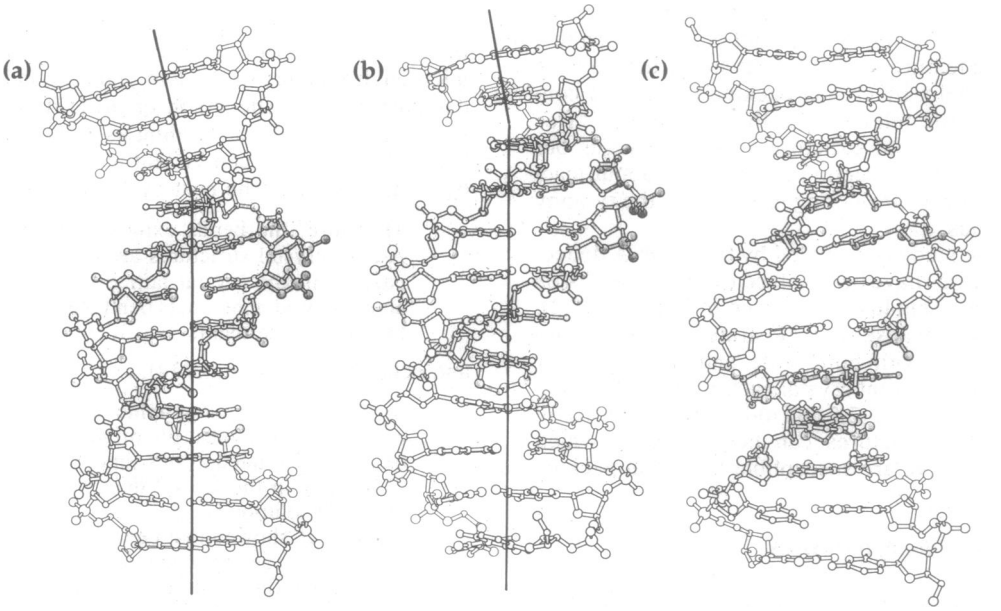

FIG. 1. Views into the minor groove of three dodecamer helices C-G-C-A-A-A-A-A-A-G-C-G (*a*), C-G-C-G-A-A-A-A-A-A-C-G or C-G-T-T-T-T-T-T-C-G-C-G (*b*), and C-G-C-A-A-G-C-T-G-G-C-C (*c*). A·T base pairs are shaded. If we keep the numbering of the original authors, base pair 1 is at the top of *a* and *c* and at the bottom of *b*. Base pair 12 is at the bottom of *a* and *c* and at the top of *b*. Note the bend to the left at the top of *a* and *b* and the straightness of the helix axis for *c*. Crystal packing is identical in *a* and *c*, so the difference in bending behavior cannot be attributed to lattice forces. Conversely, crystal packing is different in *a* and *b*, so the similarity of bending at the top of each helix cannot be lattice-induced. In both *a* and *b*, bending occurs at a junction between regions of G·C and A·T base pairs, by rolling base pairs about their long axes in a direction that compresses the wide major groove. Note the narrow minor groove in *a* and *b* and the wide minor groove in *c*, demonstrating that minor-groove width is not simply a consequence of crystal packing forces, which are identical for *a* and *c* but different for *b*.

bend occurs at a defined end relative to the crystal, bending evidently is inducible with equal facility at the 5′ and 3′ ends of an A-A-A-A-A-T tract. Nelson *et al.* (8) reported a single orientation for C-G-C-A-A-A-A-A-A-G-C-G, with a bend at the 5′ end of A-A-A-A-A-A but not the 3′ end. DiGabriele and Steitz (9) found that a monobrominated double helix of the same sequence exhibited end-for-end disorder within the crystal like that with A-A-A-A-A-T, again indicating no difference in bending behavior between the 5′ and 3′ ends of an A-A-A-A-A-A tract.

Bromination of cytosine at the 5 position within the major groove is sufficient to remove the 19° bend at the upper end of the helix in C-G-C-G-A-A-T-T-^{5br}C-G-C-G (10). Although this structure is isomorphous with the other dodecamers discussed so far, its helix is straight and unbent. Evidently the introduction of so small a group as a bromine atom within the compressed major groove is sufficient to push the top of the helix back and remove the bend, in spite of local crystal packing forces.

In all examples so far, the bend occurs at a junction between G·C and A·T regions of sequence. But note that this observed GC/AT junction bend is not what is demanded by the conventional junction model for A-tract bending (16, 17). In the quotation given above, Koo *et al.* (14) state that the direction of bending in the crystal does not conform to the results in solution. What they should have said was that the direction of bending in the crystal does not conform to *their assumed model regarding bending in solution*: the junction–bend model. That model requires a change of tilt, or of inclination of base pairs to the helix axis, at a GC/AT junction. This is 90° away from what is seen in all of the crystal structures of Table 1, where bending is produced by a pure roll motion.

The observed dodecamer structures also provide no support for the junction model's need for a difference in base-pair inclination between A-tract and general-sequence DNA. In C-G-C-G-A-A-T-T-C-G-C-G, if a best helix axis is established through the bottom eight base pairs (omitting the tilted C-G-C-G at top), then the four A·T base pairs are inclined to the overall helix axis by a mean of −3.2°, while the four G·C pairs have a mean inclination of −3.1°. Corresponding inclinations for A-tract and G·C regions in C-G-C-A-A-A-A-A-A-G-C-G are −2.3° and −2.5°, respectively. These small differences in inclination are entirely trivial. A steeper base-pair inclination evidently is not a property of A-tract B-DNA. (At a risk of stating the obvious, A-tract B-DNA is quite distinct from A-DNA, which does exhibit a large base pair inclination.)

A Different Crystal Environment

DiGabriele and Steitz (9) successfully broke the end-for-end equivalence of crystal packing observed in C-G-C-A-A-A-A-A-T-G-C-G and in brominated C-G-C-A-A-A-A-A-A-G-C-G, by moving the A_6 tract one position down the dodecamer helix. The sequence C-G-C-G-A-A-A-A-A-A-C-G crystallizes in a new space group—$P2_12_12$—with two separate dodecamer double helices in the asymmetric unit. These helices are not disordered end-for-end. They show a bend only at the 3′ end of the A-A-A-A-A-A tract, not the 5′ end. As seen in Fig. 1*b* (see also Fig. 3*b*), the bend involves primarily base pairs 12, 11, and 10 at the top of the helix; the A-tract below it again is comparatively unbent. This bend is the same as that encountered in all of the other A-tract dodecamers: a roll bend at an AT/GC junction, compressing the major groove. DiGabriele and Steitz (9) maintain that this bend is turned 180° away from that reported in other A-tract dodecamers, presumably because it occurs at the 3′ end of the A_6 sequence (or at the 5′ end of the T_6). But this is identical to the bend in the "inverted" orientation of C-G-C-A-A-A-A-A-T-G-C-G (= C-G-C-A-T-T-T-T-T-G-C-G) and brominated C-G-C-A-A-A-A-A-A-G-C-G (= C-G-C-T-T-T-T-T-G-C-G), so the basis for their assertion of uniqueness of bend direction is not clear.

In summary, the same major-groove-compressing roll bend is encountered at the AT/GC junction in six of the seven A-tract dodecamers of Table 1, whether this junction occurs two, three, or four base-pair steps in from the end of the helix and whether the space group is $P2_12_12_1$ or $P2_12_12$. This is a facultative bend, which can occur or not, depending on outside forces. And as the C-G-C-G-A-A-T-T-^{5br}C-G-C-G dodecamer shows, these bending forces contributed by crystal packing are so weak that they can be reversed by introduction of a single bromine atom into the major groove. Moreover, there appears to be no difference between bending properties at the 5′ or 3′ end of a poly(dA) tract.

Dodecamers Without A-Tracts

The influence of crystal packing is further called into question by consideration of another B-DNA dodecamer structure with a quite different sequence (Table 1, lower part). C-G-C-A-A-G-C-T-G-G-C-G has two A·G mispairings that are irrelevant to the present discussion. But it also lacks a central A-tract flanked by GC/AT junctions. Although it crystallizes in exactly the same orthorhombic lattice as the first six dodecamers of Table 1, upper part, it displays no bend in the upper third where five of the other six helices are bent (Fig. 1*c*). Hence crystal lattice forces, although facilitative if a step such as an AT/GC junction happens to be a natural hinge, cannot force a bend where no such hinge exists. This is unsurprising, since the intermolecular interactions involved in these crystals are largely the relatively weak hydrogen bonds.

The sequence C-G-C-A-A-G-C-T-G-G-C-C does have a bend, but the bend occurs at the G-C step in the very center and in a direction at right angles to that found in the A-tract dodecamers of Table 1, upper part. This bend can be seen in Fig. 2*c* and in the normal-vector plot of Fig. 3*c*. As with the A-tract dodecamers, the bend takes place by a pure rolling motion, in a direction that compresses the wide major groove. But the two types of bend occur in different directions because the bend centers are located at different places down the length of the dodecamer.

So, the particular crystal packing encountered by these dodecamers in orthorhombic space group $P2_12_12_1$ can accommodate (*i*) a bend in one plane in the vicinity of base pair 3, (*ii*) a bend at right angles to this between base pairs 5 and 6, or (*iii*) no bend in helix axis at all. Crystal packing *per se* demands none of these three situations. Nor is it correct to maintain that the straight and unbent A-tracts in the middle of the sequences of Table 1, upper part, are held that way by crystal packing. If this were true, then bent C-G-C-A-A-G-C-T-G-G-C-C would also have been held straight by the lattice. Conversely, if A-tracts *did* possess any inherent tendency to bend, the orthorhombic crystal lattice of the dodecamers could have accommodated that bend, because such a bend actually is seen with a different base sequence. The fact that the center of the sequence C-G-C-A-A-A-A-A-A-G-C-G is straight, whereas in an identical crystal environment the center of the sequence C-G-C-A-A-G-C-T-G-G-C-C is bent, can mean only that A-tracts have an inherently smaller tendency to bend than does a G-C step. This, incidentally, agrees with recent crystal structure analyses of B-DNA decamers that reveal an inherent tendency toward major-groove-compressing roll bending at the sequence G-G-C-C (18, 19). Note that in every observed example of bending in B-DNA, the bend occurs by rolling one base pair over another along their long axes. A bend involving tilt, or change in base-pair inclination, is never seen.

These two dodecamers without A-tracts also illustrate another crystal-independent structural feature, involving minor-groove width and base-pair propeller twist. As columns

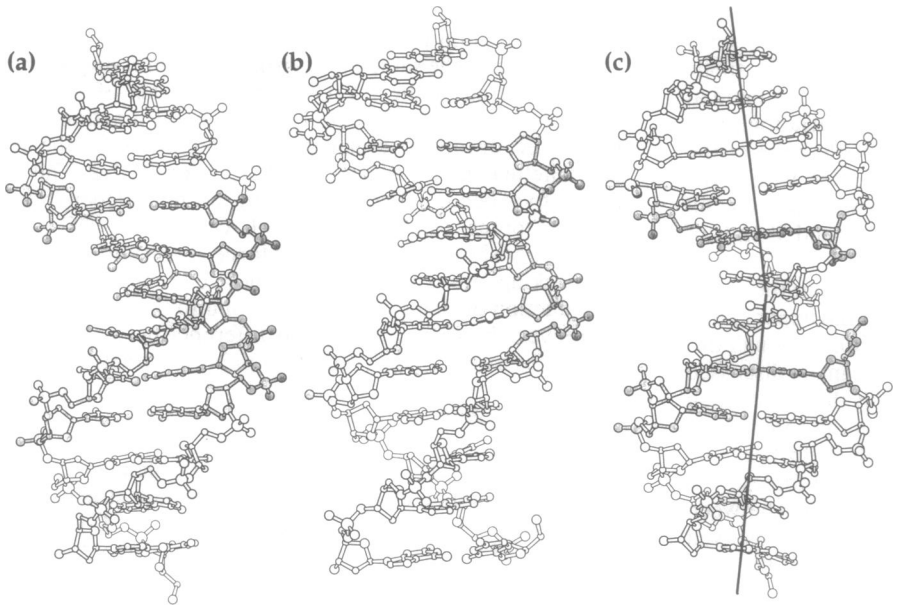

FIG. 2. Views from the left of Fig. 1, for the same three helices as shown in Fig. 1. Note now that the helix axis is straight in this plane for *a* and *b*, but is bent for *c*. For magnitudes of bends, see the normal-vector plots in Fig. 3. As always, the bend involves a rolling of base pairs around their long axes and compression of the major groove. Although crystals of helices *a* and *c* are isomorphous, the bends seen in Fig. 1*a* and here in *c* occur in different directions in the crystal and at different locations along the dodecamer. Hence the bends cannot be simple crystal artifacts.

Y and Z of Table 1 show, A-tracts in these dodecamers universally exhibit narrowed minor grooves and large base-pair propeller twist. The structural reason for this correlation of groove width and propeller twist is demonstrated by figure 9 of ref. 10. Sequences other than pure A-tracts, such as -T-A-G-A-T-C-T-A-, also can have a narrow minor groove, which is found with various sequences in three entirely different space groups and crystal packing modes: $P2_12_12_1$, $P2_12_12$, and $C2$. By contrast, another non-A-tract sequence, C-G-C-A-A-G-C-T-G-G-C-G, exhibits a wide minor groove and flattened propeller twist, even though it is packed into a $P2_12_12_1$ unit cell in exactly the same manner as the narrow-groove A-tract dodecamers (Table 1). Hence, for these particular dodecamer sequences and space groups, the width of the minor groove is not determined by intermolecular crystal packing, leaving base sequence as the only remaining determining factor.

Sequence-Dependent Differential Deformability

Many other local helix parameters, of course, are strongly influenced by local environmental forces, whether these arise from adjacent DNA molecules in a crystal lattice or from a bound repressor, transcription factor, or other control protein. DNA has evolved to interact with other macromolecules, and a free DNA helix in solution may in fact be the least biologically relevant state of all. It is clear that many sequences are capable of more than one state of local variables such as twist, rise, and roll. Leonard and Hunter (12) have recently pointed out the weakness of any "one-sequence/one-state" picture of DNA structure. A paper published in 1982 was entitled "The ten helical twist angles of B-DNA" (20). It has been amply demonstrated since that time that DNA does not have 10 standard helical twists corresponding to the 10 possible base-pair steps in a self-complementary double helix. Calladine (21) and Dickerson (22) improved the simple base-step model to allow for the influence of adjacent base steps, and Yanagi *et al.* (23) in 1991 tried to extend the analysis systematically to all 136 possible tetrad sequences: regions of three successive steps or four successive base pairs. Subsequent studies have demonstrated that B-DNA is more variable than these simple static models would predict. The concept of 136

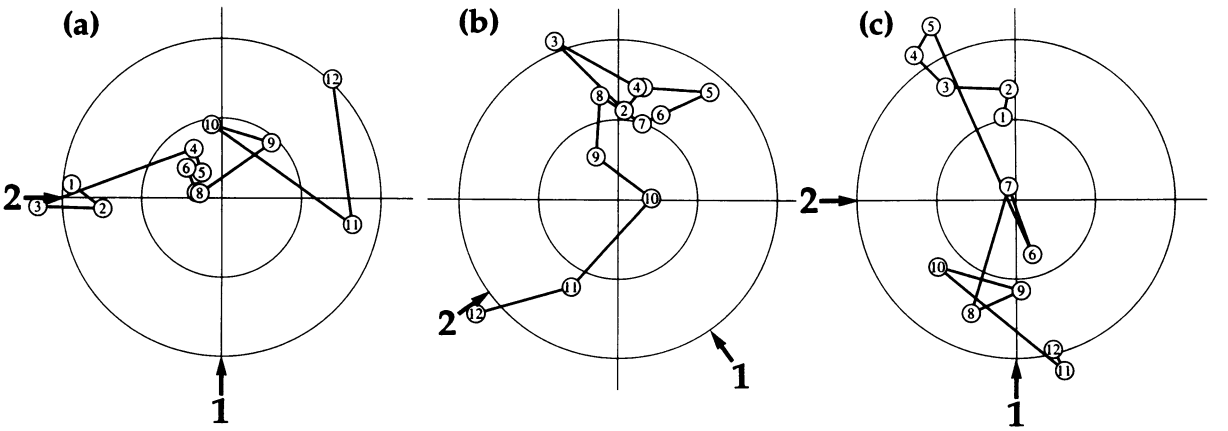

FIG. 3. Normal-vector plots for the three helices of Figs. 1 and 2: C^1-G-C-A-A-A-A-A-A-G-C-G^{12} (*a*), C^{12}-G-T-T-T-T-T-T-C-G-C-G^1 (*b*), and C^1-G-C-A-A-G-C-T-G-G-C-C^{12} (*c*). A normal-vector plot is produced by generating a vector perpendicular to each base pair, bringing all these vectors to a common origin, and then viewing the ensemble of vectors down the helix axis from the top. Each numbered circle, 1–12, represents the tip of the vector belonging to the base pair of the same number. The arrow labeled 1 in each plot indicates the direction of view in Fig. 1 *a–c*, and arrow 2 indicates the viewing direction in Fig. 2 *a–c*. Viewing directions 1 and 2 are rotated 36° in *b* because the bend occurs one base pair closer to the upper end of the helix. A sequential progression of numbered circles across the plot indicates an overall bend in the helix. Inner and outer rings in the plots correspond to 5° and 10°, respectively. Note the left-to-right bend in *a* and *b* and the front-to-back bend in *c*, demonstrating that the bends are not simply a consequence of crystal packing forces, which are identical for helices *a* and *c* but different for *b*.

standard tetrads is as erroneous as is that of 10 standard steps. DNA is inherently locally polymorphous.

Having said this, we must add that all evidence to date suggests that DNA is not *randomly* polymorphous. Generalizations can be drawn—generalizations that of course are subject to modification by the next crystal structure published but which begin to carry a ring of truth because of their repeated verification in many structures. These include the straightness of poly(dA) tracts; the tendency of regions of A·T base pairs to exhibit a narrow minor groove, large propeller, and minor groove spine of hydration; the propensity of Y-C-A-R steps for large twist and slide (23); roll bending in G·C regions, especially at G-G-C-C steps (18, 19); and a major-groove-compressing roll bend at T-A (24).

The following conclusions about the properties of B-DNA are deduced for the dodecamers of Table 1.

(*a*) A-tracts are inherently straight and unbent.§
(*b*) The mean base-pair inclination in A-tracts is no different from that in general-sequence B-DNA.
(*c*) Junctions between A-tract and general-sequence B-DNA constitute a flexible hinge, at which bending occurs by a pure roll motion, with no tilt component, and most easily in a direction that compresses the major groove.
(*d*) The sequence A-G-C-T also is compatible with a roll bend compressing the major groove.
(*e*) Crystal packing forces in the orthorhombic $P2_12_12_1$ lattice of the dodecamers of Table 1 *permit* either of the two bends enumerated in *c* and *d* but *require* neither. Hence intermolecular forces, in this instance, are of secondary importance.
(*f*) The narrow minor groove found in the dodecamers of Table 1, upper part, is not imposed by the crystal lattice, and therefore must be a characteristic of A-tracts.
(*g*) A narrow minor groove is correlated with large base-pair propeller and with a single spine of hydration down the center of the groove.

It should be noted in passing that conclusion *a* above falsifies the bent-A model for A-tract bending, whereas conclusions *b* and *c* invalidate the junction model. Conclusions *a–d* are all compatible with the only remaining choice, the bent non-A model (18, 19, 24).

In sum, different sequences exhibit different tendencies to twist, roll, or bend, and these tendencies surely constitute part of the recognition process by proteins and drugs. The DNA double helix is not a rigid structure, but neither is it a formless mass of spaghetti. A better analogy might be that of a human arm, which at different points along its length exhibits different propensities toward bending and rotation. The sequence of the DNA double helix influences the way the helix behaves in the same way that the local structure of the arm determines whether it will flex, bend, rotate, or resist bending at each point along its length. It is wrong to maintain that crystal packing forces have no effect on local helix structure. It is even more wrong, however, to write off the experimentally observed sequence effects entirely and to maintain that local helix structure is entirely at the mercy of crystal forces. One must examine a related series of structures, and different local crystal environments, to ascertain the relative influence of base sequence and crystal packing forces for a particular DNA sequence or type of DNA. The fact that we can observe some sequences in different crystal environments gives us the opportunity to visualize directly the nature and extent of sequence-directed polymorphism, a polymorphism that needs to be explained rather than being dismissed out of hand as "crystal artifacts." The set of B-DNA dodecamers has constituted one such case study. In the two cases just considered, involving helix bending and minor groove width, the classical verdict "sic semper *tyrannis*" is somewhat Brutal. The tyrant in fact is a constitutional monarch, subject to the constitution of the base sequence.

§This universal straightness of A-tracts in the crystal is emphasized strongly by DiGabriele and Steitz (9). They close their paper with the query, "Why are adenine tracts straight in crystals, and why do they appear bent in solution?" They first attempt to rationalize these observations by proposing that A-tracts are prevented from bending in crystal by the spine of hydration and that this spine is frozen in place by the 30% methylpentanediol necessary to produce crystals. But ultimately they conclude, as do we, that: "Alternatively, the observation of the spine of hydration in aqueous solution [from NMR, (20)] and the results of x-ray crystallographic studies *may require a reinterpretation of the structure of A-tract DNA derived from solution experiments*" (emphasis added).

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1. Wing, R., Drew, H., Takano, T., Broka, C., Tanaka, S., Itakura, K. & Dickerson, R. E. (1980) *Nature (London)* **287,** 755–758.
2. Dickerson, R. E. & Drew, H. R. (1981) *J. Mol. Biol.* **149,** 7671–7686.
3. Larsen, T. A., Kopka, M. L. & Dickerson, R. E. (1991) *Biochemistry* **30,** 4443–4449.
4. Narayana, N., Ginell, S. L., Russu, I. M. & Berman, H. M. (1991) *Biochemistry* **30,** 4449–4455.
5. Coll, M., Frederick, C. A., Wang, A. H.-J. & Rich, A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8385–8389.
6. Edwards, K. J., Brown, D. G., Spink, N., Skelly, J. V. & Neidle, S. (1992) *J. Mol. Biol.* **226,** 1161–1173.
7. DiGabriele, A. D., Sanderson, M. R. & Steitz, T. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1816–1820.
8. Nelson, H. C. M., Finch, J. T., Luisi, B. F. & Klug, A. (1987) *Nature (London)* **330,** 221–226.
9. DiGabriele, A. D. & Steitz, T. A. (1993) *J. Mol. Biol.* **231,** 1024–1039.
10. Fratini, A. V., Kopka, M. L., Drew, H. R. & Dickerson, R. E. (1982) *J. Biol. Chem.* **257,** 14686–14707.
11. Webster, G. D., Sanderson, M. R., Skelly, J. V., Neidle, S., Swann, P. F., Li, B. F. & Tickle, I. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6693–6697.
12. Leonard, G. A. & Hunter, W. N. (1993) *J. Mol. Biol.* **234,** 198–208.
13. Dickerson, R. E., Goodsell, D. S., Kopka, M. L. & Pjura, P. E. (1987) *J. Biomol. Struct. Dyn.* **5,** 557–579.
14. Koo, H.-S., Drak, J., Rice, J. A. & Crothers, D. M. (1990) *Biochemistry* **29,** 4227–4234.
15. Kunkel, G. R. & Martinson, H. A. (1981) *Nucleic Acids Res.* **9,** 6869–6888.
16. Crothers, D. M., Drak, J., Kahn, J. D. & Levene, S. D. (1992) *Methods Enzymol.* **212,** 3–29.
17. Crothers, D. M. & Drak, J. (1992) *Methods Enzymol.* **212,** 46–71.
18. Goodsell, D. S., Kopka, M. L., Cascio, D. & Dickerson, R. E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2930–2934.
19. Grzeskowiak, K., Goodsell, D. S., Kaczor-Grzeskowiak, M., Cascio, D. & Dickerson, R. E. (1993) *Biochemistry* **32,** 8923–8931.
20. Kabsch, W., Sander, C. & Trifonov, E. N. (1982) *Nucleic Acids Res.* **10,** 1097–1104.
21. Calladine, C. R. (1982) *J. Mol. Biol.* **161,** 343–352.
22. Dickerson, R. E. (1983) *J. Mol. Biol.* **166,** 419–441.
23. Yanagi, K., Privé, G. G. & Dickerson, R. E. (1991) *J. Mol. Biol.* **217,** 201–214.
24. Goodsell, D. S., Kaczor-Grzeskowiak, M. & Dickerson, R. E. (1994) *J. Mol. Biol.*, in press.