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Structure of a B-DNA dodecamer at 16 K*

(low-temperature crystallography/molecular disorder vs. vibration/crystalline librational disorder/DNA at ultralow temperature)

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ABSTRACT **The crystal structure of the B-DNA dodecamer C-G-C-G-A-A-T-T-C-G-C-G, previously solved and refined at room temperature (290 K), has been analyzed at 16 K (−257°C). The end-to-end winding of the helix does not vary with temperature but remains constant at 10.1 base pairs per turn. Negatively charged phosphate groups throughout the structure do move closer together on cooling, however, probably because of increase in the dielectric constant of water as the temperature is lowered. This has the two-fold effect of reducing the spacing between neighboring double helices from 24.0 to 22.9 Å and of narrowing the helix grooves within any isolated molecule. Overall lattice displacements as deduced from crystallographic temperature factors are very much decreased in the 16 K structure, yet displacements at phosphates continue to exceed those of deoxyribose sugars by B = 9 Å² and those of base pairs by B = 22 Å², even at this very low temperature at which practically all thermal motion has been eliminated. These differences, formerly interpreted as evidence for thermal vibration, must now be attributed to static disorder.**

Protein and nucleic acid crystallography has traditionally been limited to temperatures of 260 K (−13°C) or higher because of the high solvent content of the crystals. Although low-temperature data collection would be valuable as a way of reducing x-ray decay in sensitive materials, as well as for directly assaying for temperature-dependent conformational flexibility in proteins (1), attempts to cool protein crystals to liquid nitrogen temperatures (ca. 77 K) have always ended in failure (2). When the liquid water channels between molecules in a protein crystal are allowed to freeze, groups of molecules in the crystal break up into slightly misaligned microcrystallites that diffract x-rays diffusely if at all. It has never been clear whether the unwanted peak broadening is due to thermal strain, internal ice formation, or a combination of these and other factors (2).

Petsko (3) has introduced a method to replace the content of these solvent channels by mixed water/alcohol "antifreeze" solutions that remain liquid to <200 K and do not denature the protein. Several protein-structure determinations have been carried out using this technique, including metmyoglobin at 220 K (4), trypsinogen at 173 K (5), and elastase at 200 K (6). The only drawback with the current generation of mixed solvents is that they still freeze at ultralow temperatures.

An alternative strategy for low-temperature x-ray work has become feasible recently, with the development by one of us (S.S.) of a low-temperature diffractometer that has closed-cycle cooling and is capable of maintaining temperatures as low as 16 K during data collection (7). Almost as easy to operate as a conventional diffractometer, this instrument provides thermostatted cooling accurate to ±0.5 K throughout the temperature range 16 K–340 K and has already proved its worth in the analysis of small molecule structures (8, 9). Having the capability of controlled cooling for very long periods of time, one can vary the cooling rate (from 2°C/min to 1°C/wk or even slower) so as to minimize damage to crystallinity of the sample. At present, not enough experiments have been done to establish the most favorable cooling rate for macromolecules in general. Nevertheless, a 2.7 Å data set of reasonable quality has been obtained from a single crystal of the DNA dodecamer C-G-C-G-A-A-T-T-C-G-C-G (10–13) cooled over the course of 3 days from 290 K to 16 K. The crystal contains 50% solvent by weight (12), so the above cooling rate may well be applicable to protein crystals of similar hydration. The structure of the DNA itself at such a very low temperature is also of interest.

One contribution that an ultra-low-temperature crystal structure analysis can make is to differentiate between thermal motion and static disorder. The crystallographic B factor is a measure of the smearing out of atomic positions in the structure analysis. This could arise because each atom is vibrating with a certain amount of thermal motion or because equivalent atoms in adjacent molecules in the crystal do not have precisely the same relative positions. At room temperature it is difficult to differentiate the two effects. But at 16 K one can be sure that the contribution of thermal vibration has been nearly eliminated, so that the residual B values can be interpreted as static disorder.

METHODS

C-G-C-G-A-A-T-T-C-G-C-G crystallizes at room temperature from 30% 2-methyl-2,4-pentanediol in space group $P2_12_12_1$ with a = 25.3 Å, b = 40.8 Å, and c = 66.5 Å. When cooled in their normal mother liquor to 16 K, these crystals retain their $P2_12_12_1$ lattice symmetry despite reduction in all three cell lengths: a to 23.44 Å, b to 39.31 Å, and c to 65.26 Å. A representative triclinic shell of data was collected to ensure this point. The intensities from all four octants hkl, $h\bar{k}l$, $hk\bar{l}$ and $h\bar{k}\bar{l}$ were found to be symmetry equivalent after correcting for absorption effects. Data were collected with 3° ω scans (Ni-filtered CuKα) to include all of the mosaically broadened peaks 2.0–2.5° wide. No x-ray decay was detected at 16 K after 300 hr of exposure, as compared with a 30–40% decay for the same time at room temperature.

The outer limit of resolution of the data, 2.7 Å, was dictated by the strength of diffraction by the crystal. In all, 1,824 unique reflections were measured between limits of 15 and 2.7 Å. Since this is less than atomic resolution, refinement was carried out by the constrained energy methods of Jack and Levitt (14). Nineteen refinement cycles, starting from the refined 290 K coordinates, reduced the crystallographic R factor for the 16 K data from 38.4% to 21.0% (or 15.1% if only the 1,051 2-σ data are used). B values were begun at 3, 10, and 19 for bases, sugars, and phosphate groups, and were refined without restraint to mean values of −3, 11, and 17.

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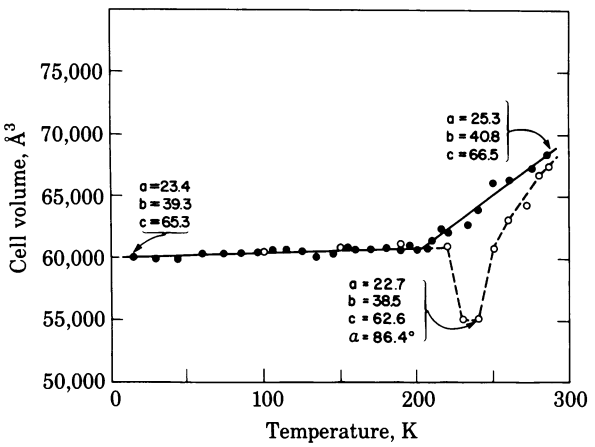

FIG. 1. Cell volume changes versus temperature for the dodecamer C-G-C-G-A-A-T-T-C-G-C-G. Lattice parameters *a*, *b*, and *c* (in units of Å) for the orthorhombic $P2_12_12_1$ cell are shown at both ends of the cooling curve. The crystal temporarily adopts monoclinic $P2_1$ symmetry on warming to 220 K and, for this cell, the *bc* angle α is 86.4°. ●, Cooling; ○, warming.

Several combined Fourier/difference Fourier maps were plotted during the course of refinement to check for correctness of the DNA conformation and to identify likely solvent peaks (12). The final phasing model contains 486 DNA atoms (one 12-base-pair double helix) and 83 discrete solvent peaks per asymmetric unit. As with all other DNA structures from this laboratory, observed and calculated structure factors, calculated phases, atomic coordinates, and individual isotropic temperature factors have been deposited with the Brookhaven Protein Data Bank.

RESULTS

Effect of Cooling on Crystal Packing. Since the C-G-C-G-A-A-T-T-C-G-C-G double helix retains much of its solution hydration even in the crystal (12), one would expect the volume of the unit cell to increase slightly on cooling because the water inside the channels between helices freezes to the less dense ice I form (0.93 g/cm^3). Quite surprisingly, the opposite occurs; the cell volume decreases substantially in the 290 K to 200 K range and then less so until 16 K is reached. This break in the curve at 200 K marks what we shall term the solvent solidification point, leaving open the question of whether this represents a true phase change. Most of the decrease, from 68,000 Å^3 to 61,000 Å^3, arises from shrinkage in the *a* and *b* directions and reflects the closer lateral spacing of the molecules. When double helices pack at center and corners of an orthorhombic cell as cylinders with their long axes oriented along the *c* direction, their lateral separations or "packing diameters" are given by half the *ab* face diagonal, $\frac{1}{2}(a^2 + b^2)^{1/2}$, where *a* and *b* are cell lengths. The calculated packing diameter at 290 K is 24.0 Å and that at 16 K is 22.9 Å. In retrospect, this shrinkage might well have been anticipated: the dielectric (and hence charge shielding) constants of water and mixed alcohol/water solutions increase as the temperature is lowered (3, 15), allowing negatively charged DNA phosphates from neighboring molecules to move closer together.

The 24 hr used to cool the crystal from 290 K to 200 K apparently were not sufficient to allow crystal packing to equilibrate fully. Subsequent cooling below this solidification point (to 16 K) produced little change in cell volume. But when the crystal was warmed after collection of a 16 K data set (open circles in Fig. 1), the reversibility of cell volume changes failed above 210 K. Instead, the volume decreased sharply from 61,000 Å^3 to 55,000 Å^3, and the space group symmetry changed

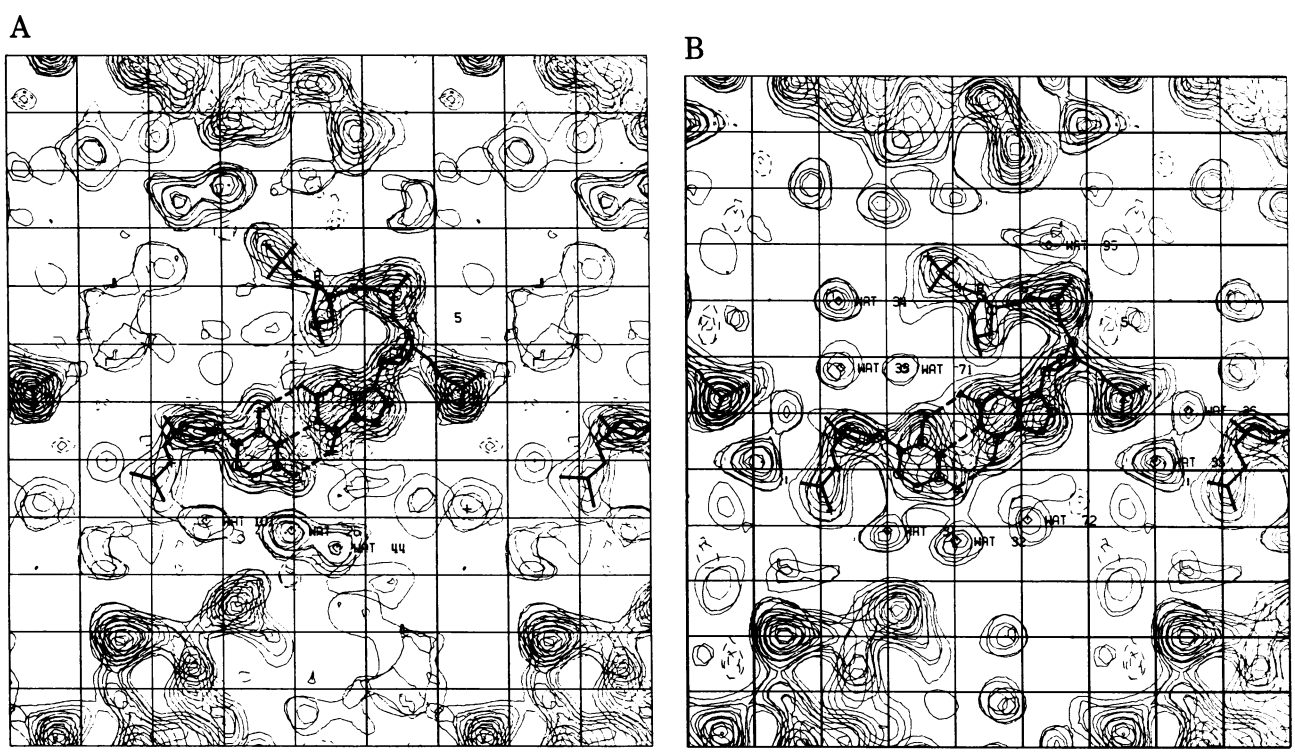

FIG. 2. Superposition of sections +23/96 through +26/96 of the 290 K (*A*) and 16 K (*B*) dodecamer electron density maps. Fewer data were used to construct the 16 K map (only 1,824 *hkl* as compared with 5,534 at 290 K), but the lower overall lattice disorder in the 16 K structure helps to sharpen the image of the DNA. Note also the more ordered solvent density and the closer packing of double helices in *B* as compared with *A*. The unit cell repeat along the horizontal *a* axis is visible at left and right, and the vertical direction shows exactly one cell repeat in *b*.

from orthorhombic ($P2_12_12_1$) to monoclinic (probably $P2_1$). Further warming (from 220 K to 280 K) restored both the original 68,000-Å^3 unit cell and the orthorhombic symmetry. It is still unclear whether much slower cooling from 290 K to 200 K at the outset would have resulted directly in the dense 55,000-Å^3 monoclinic cell. The present 16 K structure may represent a supercooled rather than a true equilibrium state, but more experiments are needed to test this.

Peak broadening, the usual menace of low-temperature x-ray work, set in at 240 K and became worse until 200 K was reached. Below this solidification point, little change was observed in the crystal, down to its final 16 K geometry. Peaks at $2\theta = 30°$, for example, widened from 0.5° at 240 K to 2.0°–2.5° at 200 K, and peak heights fell accordingly. These observations suggest that the limits 240 K and 200 K may represent the beginning and end, respectively, of the solvent freezing transition. The undesirable mosaicity was not reversed on rewarming and persisted after reheating to 290 K.

This peak broadening lowered the signal-to-noise ratio at high θ angles, and effectively limited the resolution to 2.7 Å, as compared with 2.0 Å for identical crystals at room temperature that had not undergone thermal cycling (10). But the loss of resolution associated with cooling was to some extent compensated for by a lower overall disorder at 16 K, 0.44 Å rms versus 0.68 Å rms at 290 K (Wilson plot values of B = 15 and 36 Å^2, respectively, as described below). Consequently, the definition or resolution of structural features in the 16 K electron density function is better than might have been expected. Composite slices through a few sections of each map, at 290 K and at 16 K, are shown in Fig. 2. Water positions around the phosphates are especially well ordered at 16 K and provide valuable information as to the role of water in B-helix stability (M. L. Kopka, A. V. Fratini, H. R. Drew, and R. E. Dickerson, unpublished).

Effect of Cooling on Molecular Structure. The refined structure of C-G-C-G-A-A-T-T-C-G-C-G at 16 K is shown in Fig. 3. The conformation of the molecule at 16 K is very similar to that at room temperature (10): two single strands containing 12 nucleotides each, twisted around each other in an anti-parallel fashion to form a classical Watson–Crick B-DNA double helix with 10.1 base pairs per helix turn. The average rotation per base step is 360°/10.1 = 35.9°, with local variations from 28° to 40° arising from the heterogeneous base sequence (11). After correction for changes in the distances between dodecamers, coordinates of the helix at 16 K differ by only 0.37 Å rms from those at 290 K. Most of the significant structural differences are found in the G-A-A-T-T-C center of the duplex. In going from 290 K to 16 K, both the minor and the major grooves of the helix close slightly in this region, by 0.6 and 0.3 Å, respectively. Groove widths, defined as the interstrand P-P distance minus 5.8 Å, decrease from 4.1 to 3.5 Å across the minor groove and from 11.5 to 11.2 Å across the major groove. Base pairs A6·T19 through C9·G16 show a larger mean propeller twist about their long axes, 23° instead of 17°. Base pair G10·C15, as if responding in a domino-like fashion to motions in the base pairs above it, increases its propeller twist from 5° to 10°.

The three-dimensional motions involved can be visualized with the help of a least-squares superposition of the two structures (Fig. 4). During cooling from 290 K to 16 K, many of the phosphate groups on the left strand move downward slightly,

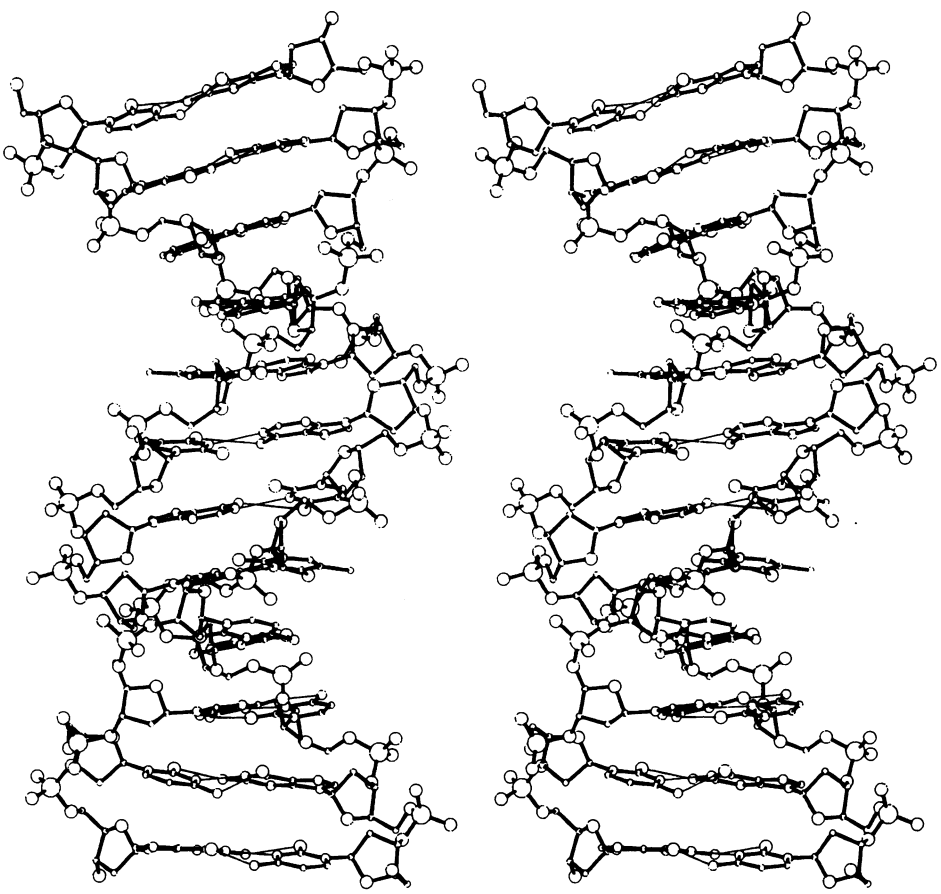

FIG. 3. Stereodiagram of the B-DNA dodecamer double helix at 16 K. Atoms in order of decreasing radius are P, O, N, C. The 5′ end of strand 1 (bases 1–12) and the 3′ end of strand 2 (bases 13–24) appear at upper left and right, respectively. Base pair C1·G24 is at the top, and G12·C13 is at the bottom. Compare this figure with the 290 K structure (figure 1*A* of ref. 12).

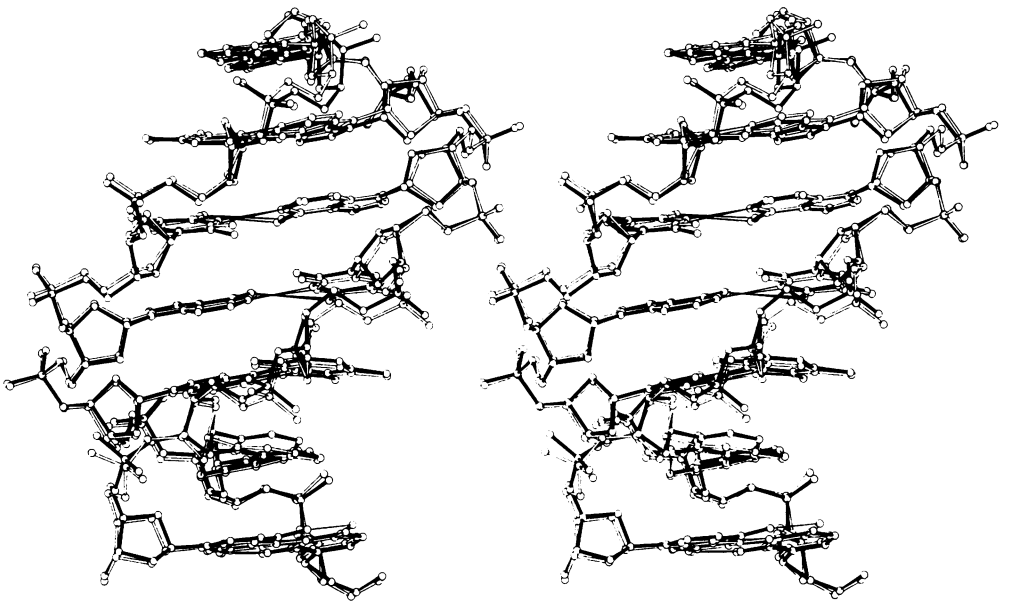

FIG. 4. Closeup of a least-squares superposition of the 290 K (light bonds) and 16 K (dark bonds) structures. Only the central G-A-A-T-T-C-G sequence is shown, with base pair G4·C21 at the top and G10·C15 at the bottom. The viewing direction is into the center of the minor groove, as for Fig. 3.

while those on the right strand move upward, narrowing the interstrand separation by 0.6 Å. As phosphates move up and down, they pull adjacent sugar rings with them, causing bases on the left strand to expose more of their upper surfaces to the viewer and those on the right to show more of their lower surfaces and increasing the propeller twist of the base pair. This coupled base–sugar–phosphate motion will be described elsewhere in more detail (A. V. Fratini, M. L. Kopka, H. E. Drew, and R. E. Dickerson, unpublished) and is of general significance in understanding the kinematics of B-DNA: narrowed minor grooves are accompanied by larger propeller twists (skewed base pairs), while wider minor grooves are associated with smaller propeller twists (flatter base pairs).

A possible explanation for the change in dodecamer structure with cooling is that the enhanced dielectric constant of water at low temperatures may alleviate unfavorable phosphate-phosphate repulsions across the minor groove and permit the groove to narrow somewhat (as well as permitting adjacent helices to pack more tightly together). If this idea is correct, then the minor groove of a B helix should open slightly as the temperature is raised, the base pairs should flatten out, and the bases themselves should approach more nearly the ideal state of perpendicularity to the helix axis. Patel has studied C-G-C-G-A-A-T-T-C-G-C-G in solution by NMR spectroscopy and does observe a temperature-dependent conformational change in the four central A·T base pairs prior to melting (16).

Disorder and Its Origins. As mentioned above, overall B values and the corresponding rms displacements at 16 K are substantially smaller than those observed at 290 K, 0.44 versus 0.68 Å rms. These averaged values, however, convey no information about the actual three-dimensional distribution of displacements throughout the molecule. In general, structural disorders are of three types: translational, librational, and conformational (17). Translational rigid-body disorder of the molecule as a whole typically affects all atoms in a structure equally and is a measure of the looseness of packing. Rigid-body librational or rotary disorder most affects those atoms that lie furthest from the axis of libration and is often characteristic of molecular shape [as evidenced by the examples of pyrene and hexamethylenetetramine (17)]. Unlike these first two, conformational disorder is an intramolecular property and includes contributions from all kinds of local structural heterogeneities such as mixed side-chain positions in proteins and mixed phosphate orientations in DNA; it probably corresponds more closely than either translation or libration to the true internal flexibility of the macromolecule in solution.

Rather than calculating rms atomic displacements $\langle u^2 \rangle^{1/2}$, it often is more convenient to discuss the isotropic temperature factors $B = 8\pi^2 u^2$, since these are the parameters that describe directly the falloff in atomic x-ray scattering power, $\exp[-B \sin^2(\theta/\lambda^2)]$ with Bragg angle θ (λ = wavelength). Plots of the independently refined group B factors from three dodecamer structures, native C-G-C-G-A-A-T-T-C-G-C-G at two temperatures (290 K and 16 K) and C-G-C-G-A-A-T-T-brC-G-C-G (the 9-Br structure) at 280 K are shown in Fig. 5. At each point in Fig. 5A, a phosphate B value is compared with those of the two bases bracketing that phosphate, while Fig. 5*B* compares B values from phosphates and neighboring deoxyribose sugars. Within each structural cluster, there are 22 points, one for each phosphate in the 12-base-pair dodecamer double helix.

Gross shifts in the overall B factor in going from one structure to another (up and down the diagonal lines $y = x + 22$ and $y = x + 9$) can be attributed to changes in the rigid-body translational disorder, since these changes affect every atom more or less equally. In Fig. 5A, the shift in minimum base B value from approximately +22 down to +4 in going from the native 290 K crystal to the 9-Br structure probably reflects the tighter packing of molecules and decreased solvent volume in the 9-Br unit cell (V = 62,000 Å^3 as compared with 68,000 Å^3 for the native). The negative B values in Fig. 5A for the base rings at 16 K are not physically meaningful but indicate only that disordering at such ultralow temperatures has been reduced below the experimental noise level of the data.

An observation of more fundamental significance is that B factors for the phosphates exceed those of the sugars by 9 Å^2 and those of the bases by 22 Å^2 in all three structures. While it is possible that many individual conformational changes within the helix, minimal at bases and maximal at phosphates, could produce such a result, a more likely interpretation is that of rigid-body librational disorder of the dodecamer about its long

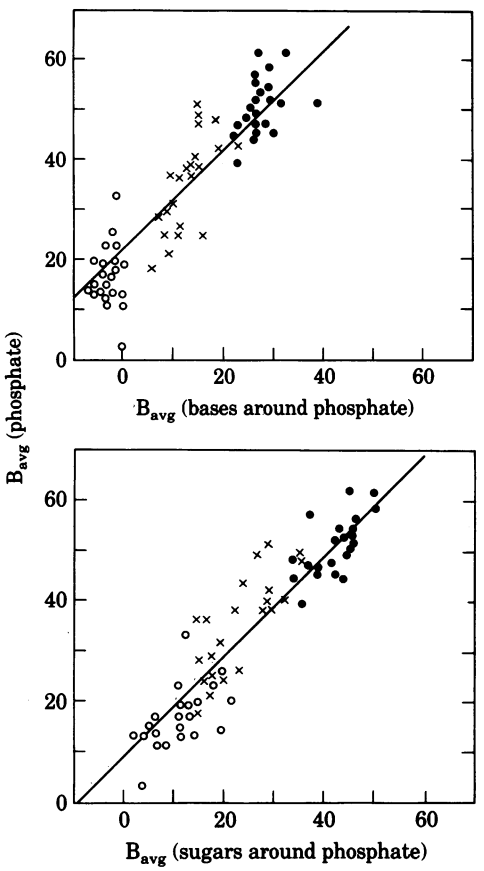

FIG. 5. Correlation plots of the group B factors in three crystalline forms of the dodecamer and its bromocytosine analogue. (*A*) Phosphates versus bases. (*B*) Phosphates versus sugars. Equations for the straight lines running diagonally across the plots are $y = x + 22$ for *A* and $y = x + 9$ for B, where "*y*" refers to phosphates and "*x*" refers either to bases or to sugars. ●, Native dodecamer at 290 K; ×, 9-Br dodecamer at 280 K; ○, native dodecamer at 16 K.

axis. We originally believed that thermally induced twisting and untwisting motions such as those displayed by DNA in solution (18) were responsible for this nonuniform distribution of displacements in the 290 K structure (10). However, we now observe that the effect persists unabated to 16 K, where thermal motion has been effectively removed, so the previous explanation seems doubtful. Librational "motions" are not uncommon in protein crystals and, in the case of the enzyme lysozyme, the same librational modes of disorder have been detected for isomers of nearly identical sequence in two different space groups, one in $P4_32_12$ and the other in $P2_12_12_1$ (19, 20).

DISCUSSION

This x-ray analysis of the dodecamer C-G-C-G-A-A-T-T-C-G-C-G at 16 K was undertaken to provide a ground-state structure for B-DNA. Solution studies have shown that thermally induced motions in the double helix produce both a large twisting–untwisting amplitude of 6° per base step (18) and a slight overall unwinding of 0.012° per base step per °C in the temperature interval 0°C to 30°C (21). The nonuniform distribution of x-ray displacements in the 290 K dodecamer structure, large at phosphates and small at base pairs, also suggested thermal motion (10). However, the present analysis leads us to believe that these displacements are primarily static rather than thermal, since they persist to 16 K where practically all thermal motion has been eliminated. Furthermore, the end-to-end twist of the dodecamer does not vary at all with temperature from its sequence-averaged value of 10.1 base pairs per turn. Taken together, these observations imply that the dynamic motions that produce twist-angle variation and helix unwinding in solution are not accessible in the crystal, even at 290 K.

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