periods of treatment, (b) well tolerated with little or no side-effects, thus increasing the compliance (acceptance and tolerance) of the patients and (c) highly effective in bringing about a significant reduction in clinical manifestations like galactorrhea and facilitating reinitiation of normal menses. The IN-BCR therapy as such appears to be a potential alternative to O-BCR in the management of hyperprolactinaemia.

A larger multicentric trial is envisaged to determine clearly the benefits of IN-BCR treatment in the management of hyperprolactinaemia. Such a study may also show if IN-BCR is beneficial in reducing the size of prolactinomas following prolonged period of treatment. Yet another use to which such kind of therapy can be adopted is in the management of long-term Parkinsonism, where patients are dependent on BCR in addition to L-DOPA throughout their life[1].

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Plasticity of Z-DNA as observed in the crystal structures of non-self-complementary hexanucleotides

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The crystal structures of two non-self-complementary hexadeoxyribonucleotides, d(CACGCG) · d(CGCGTG) and d(CGCACG) · d(CGTGCG), containing a single A : T base pair each have been solved. Both the sequences are left-handed Z-DNA. The conformation of d(CACGCG) · d(CGCGTG) is very similar to Z-DNA conformations reported earlier, while that of d(CGCACG) · d(CGTGCG) is substantially different. A shift in the position of the A : T base pair is probably responsible for inducing a change in the structure of the tightly wound Z-DNA helix. This is the first time that such large distortions have been observed in closely packed crystals of Z-DNA sequences.

SINCE the discovery, by solution spectroscopic studies[1] and single crystal X-ray diffraction[2], of left-handed Z-DNA in $d(CG)_n$ sequences, evidence has accumulated to support a possible significant biological role for this form of the genetic molecule. Among these is the discovery of regions of the eukaryotic chromosome which cross-react with antibodies raised against Z-DNA sequences[3] and the presence of Z helicogenic $(CG)_n$ sequences in the DNA of histidine D gene of *Salmonella*[4]. Repetitive (TG/CA)*n* sequences capable of adopting Z-DNA conformation have also been found to occur in human and rodent genomes[5]. Studies of the Z-DNA propensities of polynucleotides and short linear DNA fragments of different base sequences have indicated that large amounts (up to 50%) of A : T base pairs in alternating $(CG)_n$ sequences do not prevent Z-DNA formation[6]. A strict alternation of the sequence (i.e. pyrimidine–purine) has also been found neither to be necessary[7] nor sufficient[8,9] to induce Z-DNA.

High-resolution Z-DNA structures have been observed in crystallographic studies of a variety of sequences[10–12], many of them containing the features indicated above. Thus, d(CGATCG) crystallized as Z-DNA without an alternating purine–pyrimidine sequence[7]. A : T base pairs in Z-DNA have also been seen in the crystals of $d(m^5CGTAm^5CG)$[13], d(CGCATGCG)[10], d(CGTACGTACG)[14] and d(CACGTG)[15]. Experimental studies[13,15] and theoretical calculations [16] have indicated a lower stability for Z-DNA when A : T base pairs are present. The high-resolution crystal structures of Z-DNA fragments have provided some clues to rationalize this Z-DNA phobicity of A : T base pairs.

All the crystal structure studies on Z-DNA (in fact,

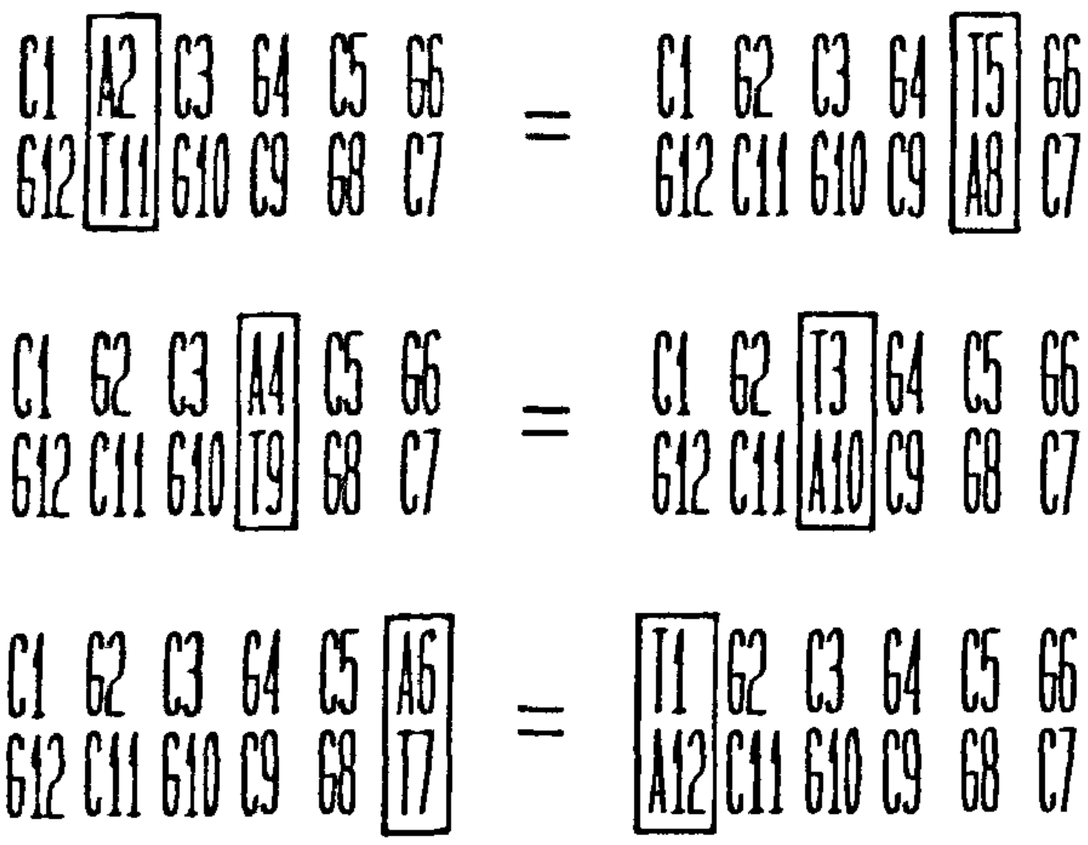

Figure 1. Three ways of inserting a single A : T base pair into the duplex d(CGCGCG) · d(CGCGCG).

most oligonucleotide crystal structure studies) have been made on self-complementary sequences. Natural DNA only rarely contains perfectly symmetrical sequences and an understanding of the structure of natural DNA is probably best achieved through studies on non-self-complementary sequences.

As an approach to this aspect of DNA structural studies and in order to make a systematic study of the nature and manner of instability that A : T base pairs would produce on the structure of Z-DNA, we undertook crystallographic studies on the effect of a single A : T base pair in the sequence. Such non-self-complementary sequences are more difficult to crystallize since this requires a careful annealing of two sequences complementary to each other before attempting to obtain crystals. The sequences we chose were based on the canonical Z-DNA hexamer[2] $d(CG)_3$. There are three ways (Figure 1) in which one could insert a single A : T base pair into the sequence in place of G : C. We were successful in obtaining crystals of the sequences d(CACGCG) · d(CGCGTG) (henceforth referred to as HA2 for hexamer with adenine at the 2nd position from 5′-end) and d(CGCACG) · d(CGTGCG) (henceforth referred to as HA4). This is the first time that crystals of non-self-complementary Z-DNA duplexes are being reported. In this paper we make a comparison of the crystal structures of these two sequences and analyse the significant distortions that a change in the position of the A : T base pair makes to the structure.

HA2 was obtained from M/s Microsynth, Switzerland, and HA4 was synthesized on an Applied Biosystems 381B automatic DNA synthesizer. Crystals of the duplexes were grown from sodium cacodylate buffer at physiological pH in the presence of $MgCl_2$ and $BaCl_2$ for HA2 and HA4, respectively. Table 1 gives the crystallographic parameters of the two duplexes. The packing of hexanucleotide helices of HA4 is almost the same as that of HA2 and of $d(CG)_3$. Nevertheless, crystals of HA4 belong to the monoclinic space group $P2_1$ rather than to the orthorhombic space group $P2_12_12_1$ seen in the crystals of other Z-DNA hexamers, including the present HA2 (ref. 17).

Refinement of the structures was carried out using the constrained Hendrickson–Konnert procedure, NUCLSQ[18], interspersed with sessions of fitting the model to the 2Fo-Fc and difference Fourier electron density maps viewed on a computer graphics system displayed using the program FRODO[19]. Since the data were available only to a resolution of 2.5 Å for HA4, the constrained molecular dynamics refinement procedure, X-PLOR[20,21], was also used for this duplex. The electron density map improved significantly after the molecular dynamics refinement.

There are no unexplained features in the final electron density maps of both the structures.

Overall, the structures of both HA2 and HA4 duplexes are within the Z-DNA family. They possess a dinucleotide repeat with an average left-handed twist of –59.5° and –57.2° per dinucleotide for HA2 and HA4, respectively. The average rise per base pair is 3.74 Å for HA2, and a somewhat lower value of 3.58 Å for HA4. The phosphate groups follow a zig-zag left-handed spiral. The helix axis passes through the deep minor groove, the major groove being almost completely flat. The alternating pyrimidine–purine bases along the sequence also alternate between the *anti* and *syn* conformation about the glycosidic bond. All the above are features of canonical Z-DNA structures[2].

Figure 2 shows the HA2 duplex superposed on $d(CG)_3$. Clearly, in the HA2 structure the presence of the A : T base pair has little effect on the Z-type conformation. The RMS deviation in the positions of the atoms after a least-squares fit[22] between the common atoms of the HA2 structure and $d(CG)_3$ is 0.49 Å. It is 0.68 Å compared to idealized ZI-DNA[23]. The bases especially show no significant perturbation of the stacking pattern. The RMS deviation of the bases alone is 0.24 Å with respect to $d(CG)_3$.

The atoms of the sugar–phosphate backbone are slightly more disturbed. The disturbance is particularly noticeable on the strand containing the adenine base. The torsion angle α about the P–O5′ bond belonging to C3 residue adjacent to the A2 residue is 179° in the present structure compared to 216° in $d(CG)_3$ and 223° in ZI-DNA. As a consequence, this portion of the backbone moves away from the ideal ZI conformation. Small changes are also noticeable in the backbone torsion angles at G4 and C5 residues in the same strand and also at G8 and C9 residues in the strand containing the thymine base.

Table 1. Crystal data, data collection statistics and refinement details for d(CGCACG) · d(CGTGCG) and d(CACGCG) · d(CGCGTG)

	d(CGCACG) · d(CGTGCG)	d(CACGCG) d(CGCGTG)
Crystal data		
Chemical formula	C_{115} H_{135} O_{68} N_{47} P_{10}	C_{115} H_{135} O_{68} N_{47} P_{10}
Molecular weight	3561	3561
Crystal size (mm)	0 5 × 0 2 × 0 2	0 3 × 0 2 × 0.2
Crystal system	Monoclinic	Orthorhombic
Space group	$P2_1$	$P2_12_12_1$
a (Å)	17 75(1)	17.76(1)
b (Å)	17 76(1)	30 96(1)
c (Å)	42.77(3)	44.75(1)
α (°)	90 0	90 0
β (°)	90 0	90 0
γ (°)	120.05(3)	90.0
V (Å^3)	11676	24606
Z	2	4
Data collection		
Diffractometer	CAD4	CAD4
Radiation type	CuKα	CuKα
Temperature (K)	296	296
No. of observed reflections	530	2625
Observation criterion	≥ 2 σ(Fo)	≥ 2 σ(Fo)
θ_{max} (°)	18	30
No. of standard reflections	3	3
Refinement		
Final *R* factor	0.161	0 199
Resolution range	10–2.5	8–1 6
No. of observed reflections used in the refinement	530	2625
Refinement method	Restrained least-squares and X-PLOR slow cooling protocol	Restrained least–squares

Tables 2 and 3 give the values of the local structural parameters in HA2, HA4 and d $(CG)_3$. It may be seen from the table that most of the base-centred parameters in HA2 are also close to the values observed in d$(CG)_3$.

The conformations of the sugars, too, do not deviate significantly from d$(CG)_3$. The average difference in the pseudorotation phase angle *P* is 14.3° between HA2 and d$(CG)_3$, while the average difference in the amplitude of pucker is 6°.

As in crystals of all Z-DNA hexamers solved[2, 9–14] (indeed, in crystals of Z-DNA sequences, no matter what the length[10, 15, 24]) the helices in the present structure stack on top of each other to form infinite fibre-like columns along the crystallographic *c* axis. Though, for hexamers crystallizing in the space group $P2_12_12_1$, the relative positions of the terminal base pairs of the helices in adjacent columns is a possible variable which would not significantly disturb the overall packing[17], a comparison of the present HA2 and d$(CG)_3$ crystal structures shows identical packing and similar intermolecular interactions. There are a few inter-helical short contacts in HA2, between the atoms of the sugar–phosphate backbones of adjacent molecules. The shortest is 2.48 Å between O1P of A2 residue of the reference hexamer and 03′ of G12 residue of the hexamer, related by a unit cell translation along the *a* axis. This distance is 2.63Å in d$(CG)_3$.

The differences between the present structure and the standard Z-DNA structures are therefore minor and the effect of the single A : T base pair in this non-self-complementary hexamer is small and may be insignificant. Where there are some small differences, these

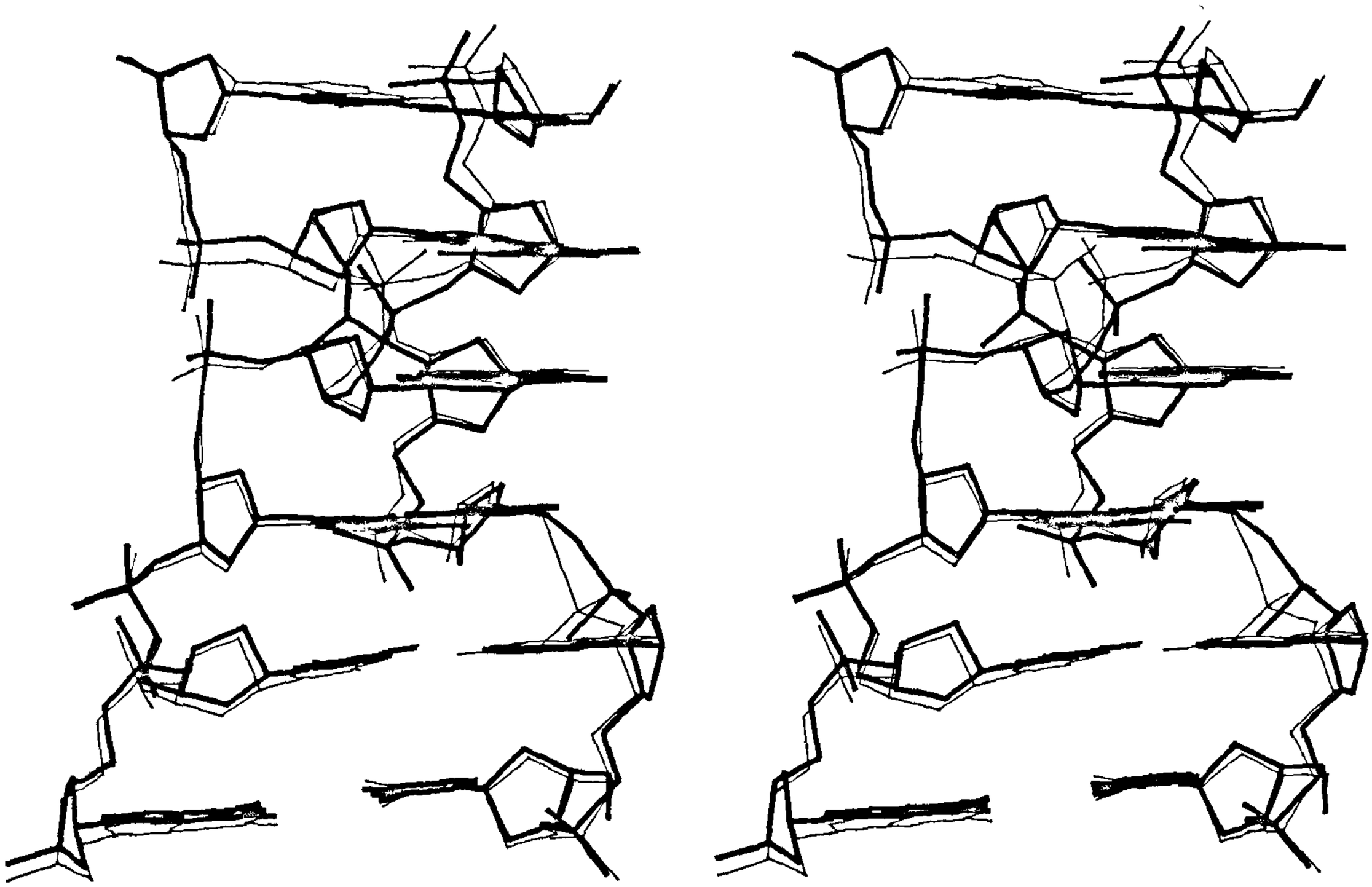

Figure 2. Undistorted Z-DNA : d(CACGCG) · d(CGCGTG) (thick lines) superimposed on d(CGCGCG) d(CGCGCG) (thin lines). (In stereo.)

are not confined to the vicinity of the A : T base pair. This is in accordance with the results obtained for other Z-DNA hexamer sequences with A : T base pairs, d(m^{5}CGTAm^{5}CG)[13], d(CGATCG)[9] and d(CACGTG)[14], which have been crystallized under a variety of conditions.

The molecule HA4 superposed on d(CG)$_3$ is shown in Figure 3. The RMS deviation of the atomic positions is 1.41 Å. In comparison with idealized ZI-DNA, the atoms deviate by an RMS value of 1.15 Å. In comparison with ZII-DNA, the deviation is larger, 1.27 Å. The HA4 helix is therefore significantly perturbed with respect to standard Z-DNA helices.

The sugar–phosphate backbone is more distorted than the bases. The RMS deviation of the positions of the atoms of the backbone from those of d(CG)$_3$ is 1.76 Å. This value is 0.84 Å for the bases. Some of the backbone torsion angles are substantially different as compared to Z-DNA values. A comparison of individual strands with corresponding strands of idealized ZI-DNA shows that the strand containing adenine deviates more than the others, with RMS values of 1.21 Å and 0.99 Å for the adenine strand and the thymine strand, respectively.

The perturbation of the structure of HA4 from regular Z-DNA is clear from the differences in the base-centred local parameters (Tables 2 and 3). The average value of the twist at the two purine–pyrimidine steps in HA4 is –42.6° as against –50.9° in d(CG)$_3$. Of the three pyrimidine–purine steps, the deviation in twist angle is much larger at the C3–p–A4 : T9–p–G10 and C5–p–G6 : C7–p–G8 steps. The values are, respectively, –24.1° and –3.3° compared to an average value of –8.4° in d(CG)$_3$. The molecule is compressed at the ends as indicated by the smaller rise at the first and last dinucleotide steps (3.38 Å and 3.24 Å as against 3.95 Å and 4.06 Å for d(CG)$_3$). Tilt values are positive at all the dinucleotide steps except at A4–p–C5 : G8–p–T9. Likewise, the values of roll are also positive at all the steps except at G2–p–C3 : G10-p-C11. In previously reported Z-DNA structures, many of the local structural parameters not only remain steady from base pair to base pair along the helix, but are also close to zero in value. HA4 violates this trend and, as may be seen in Tables 2 and 3, the values of tilt and roll are relatively large compared to both HA2 and d(CG)$_3$. In addition, the values fluctuate noticeably along the hexamer.

The ribose ring conformations in HA4 do not follow those of the previously reported Z-DNA structures. A major difference is in the conformation of the ribose

Table 2. Twist, rise, tilt, roll and slide in $d(CG)_3$, d(CACGCG) · d(CGCGTG) [HA2] and d(CGCACG) · d(CGTGCG) [HA4]

Sequence	Dinucleotide step	Twist (°)	Rise (Å)	Tilt (°)	Roll (°)	Slide (Å)
$d(CG)_3$	C1–G2 : C11–G12	–7 5	3 95	–1 6	–4 1	5.28
HA2	C1–A2 : T11–G12	–8 1	3.84	–1 6	–2.4	5.15
HA4	C1–G2 : C11–G12	–16.3	3 38	3.0	6.5	5.54
$d(CG)_3$	G2–C3 : G10–C11	–49 8	3.90	–2 8	–0.1	–0 75
HA2	A2–C3 : G10–T11	–49 4	3.75	0.6	1.5	–0.64
HA4	G2–C3 : G10–C11	–40 4	3.97	0.1	–6.1	–1.42
$d(CG)_3$	C3–G4 : C9–G10	–8 0	3.57	0.2	–4 4	5.38
HA2	C3–G4 : C9–G10	–10.3	3.65	–1.0	–3.7	5.35
HA4	C3–A4 : T9–G10	–24 1	3.78	2.7	0.4	6.38
$d(CG)_3$	G4–C5 : G8–C9	–52 0	3.65	0 2	1.7	–0.59
HA2	G4–C5 : G8–C9	–48 1	3.69	1.4	3.5	–0.75
HA4	A4–C5 : G8–T9	–44.7	3.51	–7.2	7 4	–1.50
$d(CG)_3$	C5–G6 : C7–G8	–9 6	4.06	2.3	0.7	5 07
HA2	C5–G6 : C7–G8	–13 6	3.75	–0 2	–2.7	5.50
HA4	C5–G6 : C7–G8	–3.3	3.24	5.3	3.1	3 87

Table 3. Propeller twist, buckle, inclination, tip, *x*-displacement and *y*-displacement in $d(CG)_3$, d(CACGCG) · d(CGCGTG) [HA2] and d(CGCACG) · d(CGTGCG) [HA4]

Sequence	Base pair	Propeller twist (°)	Buckle (°)	Inclination (°)	Tip (°)	*x*-dis. (Å).	*y*-dis. (Å)
$d(CG)_3$	C1 · G12	–0 5	–0.5	–8.2	7.2	2.83	–3.92
HA2	C1 · G12	–0 1	–3.2	–6 0	2.1	2 86	–3 50
HA4	C1 · G12	–7.4	5 8	2.6	–8 8	3.52	–3.70
$d(CG)_3$	G2 · C11	–3 4	6 2	–6.7	3 2	2.26	0.54
HA2	A2 · T11	–1 8	5 6	–4 5	–0.2	2.49	0.92
HA4	G2 · C11	–6 3	0.2	–0 4	–2.3	3.82	0.87
$d(CG)_3$	C3 G10	–6 0	–4 3	–3 9	3 1	1.36	–2.33
HA2	C3 · G10	–0.5	–7.4	–5.1	1.2	1.85	–2.13
HA4	C3 · G10	–2 6	11.4	–0.4	–8.4	2 92	–3.15
$d(CG)_3$	G4 · C9	–0 1	8.2	–4 1	–1.2	1.32	2 64
HA2	G4 · C9	–2.1	8 2	–4.1	–2.4	1.85	2.64
HA4	A4 · T9	–4.9	3 3	–3 1	–7 9	2 63	2.06
$d(CG)_3$	C5 · G8	0 0	0 2	–4 3	0 4	2 65	–0.22
HA2	C5 · G8	–2.0	–8 5	–5 6	1 0	3 02	–0.74
HA4	C5 · G8	–6.4	–7.3	4 1	–0.5	1.91	–1.52
$d(CG)_3$	G6 · C7	–4 8	–5 4	–6 6	1 1	2.94	3 99
HA2	C6 · C7	–3.1	3.2	–5.4	–1 6	3.14	3.74
HA4	G6 · C7	–5.4	–0.1	–1 1	2.5	2.56	2.27

in G6 residue. The value of pseudorotation phase angle P is 56°, closer to C3′-endo ($0° \leq P \leq 36°$) rather than to C2′-endo conformation ($144° \leq P \leq 180°$) seen, for example, in $d(CG)_3$. The ribose ring attached to the adenine base has a C2′-exo conformation. This again is different from the conformation of sugar attached to the corresponding guanine base in $d(CG)_3$. It may be noted that in ideal Z-DNA, sugars attached to guanine bases are in the C3′-endo conformation while those attached to cytosine bases are C2′-endo. The average difference in pseudorotation phase angles between the present structure and the crystal structure of $d(CG)_3$ is 62.6°, while the average difference in the amplitude of pucker is 11.2°.

As in the HA2 structure, the deviations of the HA4 helix from the standard Z-DNA structure are not confined to the neighbourhood of the A : T base pair. It is, however, clear that this sequence, with the single A : T base pair closer to the centre, is perturbed quite substantially from the standard Z-DNA conformation.

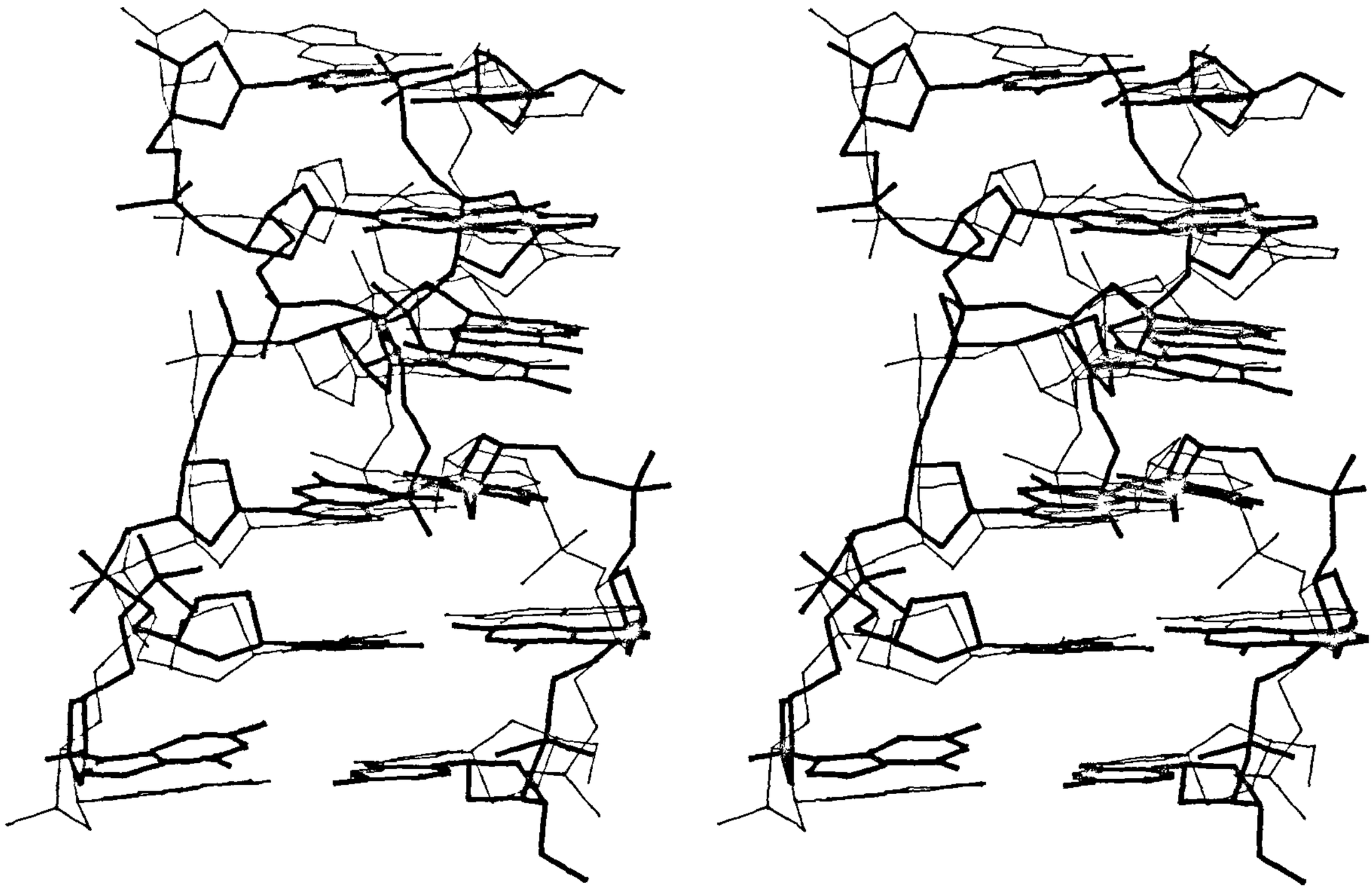

Figure 3. Distorted Z-DNA : d(CGCACG) · d(CGTGCG) (thick lines) superimposed on d(CGCGCG) · d(CGCGCG) (thin lines) (In stereo)

The packing of the HA4 helices in the crystal gives rise to a near degeneracy in the space group assignment leading to the possibility of indexing the crystal diffraction pattern in any one of the space groups $P2_1$, $P2_12_12_1$, C2 or $P6_5$. Intensity statistics dictate the choice of $P2_1$ as the correct space group[17]. While the common Z-DNA packing pattern of infinite fibre-like columns along the *c* axis persists, in the present structure the adjacent columns are related by unit cell translations along the *a* and *b* axes unlike in HA2 and $d(CG)_3$ (and several other Z-DNA crystals), where the helices in the adjacent columns along the *b* axis are related by 2_1 screw axes. This leads to contacts between neighbouring helices which are different from those observed in other Z-DNA hexamer crystals. The shortest contact, 2.58 Å, is between O4′ of A4 and O4′ of G10 of the molecule related by a unit cell translation along the *b* axis.

There is a decrease of about 2 Å in the length of the unit cell *c* axis and this leads to a reduction in the volume available to each hexamer duplex in the crystal. HA4 is thus more tightly packed than the other Z-DNA hexamers. The volume per hexamer duplex in HA4 is 5838 Å^3, compared to 6151 Å^3 in HA2 and 6284 Å^3 in $d(CG)_3$.

The tendency of DNA oligonucleotides to crystallize in a few distinct helix-type-dependent packing patterns has been noted earlier[12, 17, 25]. However, as demonstrated in the present two structures as well as in others[8, 26, 27], sequence-dependent deviations from the idealized A, B or Z type helices can be detected in the crystal structures even where the different sequences pack in the same pattern, crystallize in the same or similar unit cell and space group, and have the same or similar inter-helical interactions. Therefore, the differences observed in the present hexamers are not a consequence of crystal packing forces but may correspond to differences in sequence, i.e. the different position of the single A : T base pair. It may be noted that crystals of the sequence HA2 were grown in the presence of Mg^{2+} whilst those of the sequence HA4 had Ba^{2+} as the cation in the crystallization medium. However, this difference in the metal ion would probably not suffice to explain the differences in the structure, an inference which is supported by the fact that the crystals of $d(CG)_3$ grown in the presence of many different cations such as Mg^{2+}, Ba^{2+}, Co^{2+} (refs. 2, 29), $[Co(NH_3)_6]^{3+}$ (ref. 28), all have almost identical structures and interhelix interactions.

Though the effect of different metal ions cannot be

entirely ruled out, the significant differences between the HA2 and HA4 structures in the present case are probably due to the sequence. This implies a subtle but well-marked influence of the distance of the A : T base pair from the end of the oligonucleotide helix on the DNA conformation. At 2.5 Å resolution, accurate details of the conformational changes in HA4 are not visible. Nevertheless, a substantial and significant deviation from other Z-DNA hexamer structures indicates an inherent plasticity in the DNA molecule which is apparent even under the close-packed conditions found in Z-DNA crystals.

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Damage and repair of DNA of human epidermoid carcinoma cells X-irradiated in the presence of chlorpromazine

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X-irradiation of human epidermoid carcinoma cells (Hep-2) in the presence of the phenothiazine drug chlorpromazine (CPZ) enhances X-ray-induced cytotoxicity and chromosomal aberrations. The cytotoxicity is partly repairable during holding of the cells in the liquid before plating. The extent of double-strand breaks (DSB) produced in the DNA during these treatments was assayed by pulse field gel electrophoresis. DSBs produced in the cell X-irradiated in the presence of CPZ is the sum of the DSBs produced by X-rays and CPZ separately. Repair of DSBs after X-irradiation in the presence of CPZ is less than that after X-irradiation without CPZ.

CHLORPROMAZINE (CPZ) is a substituted phenothiazine used clinically as an antiemetic antipsychotic agent[1] and recently it is being considered as an adjuvant to cancer chemotherapy for its ability to enhance cytotoxic effects of anticancer drugs[2] and radiation[3]. We have shown that

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