Plasticity of the DNA Double Helix[1]

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Abstract: In order to determine the detailed dynamic spatial configuration of long dG-dC stretches in double-stranded DNA, magnetic shielding constants were derived for a poly(dG-dC)·poly(dG-dC) double helix from the x, y, and z coordinates of A-DNA, alternating B-DNA, Arnott and Hukins' B-DNA, C-DNA, D-DNA, the vertical double helix, the Z1-DNA, the Z2-DNA, and Levitt and Dickerson's propeller twist DNA's taking into account the contribution to shielding from the diamagnetic and paramagnetic components of atomic magnetic anisotropy, as well as the ring-current effects. The computed shielding values for all the forms were examined vis-a-vis the experimentally observed solution nuclear magnetic resonance shift data for poly(dG-dC)·poly(dG-dC) in high and low salt solutions. Among the ten different spatial configurations examined, the data indicate that in high salt solution the time-average structure is essentially identical with Z1-DNA, except that local fluctuations about dC is such that the χ_{CN} of dC in the solution structure is about 24–25° rather than the 21° in the x, y, and z of Z1-DNA. It is further shown that the time-average solution structure is very close to the real structure with finite lifetime, and it is not the average of some widely different spatial configurations in the fluctuation itinerary. In low salt solution at 81 °C, a temperature 8 °C below the onset of melting, the structure is best described as an equilibrium between high populations of Arnott–Hukins' B-DNA and a model in which the dG may adopt a syn conformation. In the Arnott–Hukins B-DNA, under our conditions, the base pairs could be either flat or mildly propeller twisted. These results along with the recent observations of Patel, Sarma, and their co-workers that in solution the AATT stretches of self-complementary d-CGCGAATTCGCG assume pronounced propeller twists a la Levitt, Crothers, and Dickerson, the findings of Sarma et al. that changing χ_{CN} from 80 to 120° causes a right- to left-handed transformation, and the revelation by Rich et al. and Felsenfeld et al. that methylation of the bases promotes B → Z transition clearly suggest the rich plasticity in the structure of the DNA double helix and its ability to assume sequence and ionic strength dependent distinct spatial configurations. This vivid demonstration of the plasticity denotes the necessity of paying serious attention to the concept of long-range allosteric transformations in DNA, propounded by Crothers, as a mechanism for the control of genomic expression.

The blueprints of the mechanism of life are preserved in genomic DNA. Little do we know about the three-dimensional dynamic solution geometry of any genomic DNA and how the structure controls expression. There have been a plethora of studies on model systems. Fiber diffraction studies in which the atoms are poorly resolved, single crystal crystallographic studies of small DNA fragments, and theoretical studies have led to the suggestion that DNA can exist in diverse spatial configurations, and one does not know how valid are these structures derived from solid state and theoretical studies for polynucleotide double helices in solution. In the present work, we undertake a systematic examination of the solution structure of the double helix poly(dG-dC)·poly(dG-dC) under high and low salt conditions.

Pohl and Jovin[2] demonstrated that poly(dG-dC)·poly(dG-dC) undergoes a salt-dependent conformational transition. They showed that at low salt concentrations the circular dichroism spectra of poly(dG-dC)·poly(dG-dC) and DNA with high dG + dC content are similar and the spectra of the synthetic duplex undergo inversion at 4 M NaCl; such inversions can be caused by the addition of mitomycin[3] or changing to a solvent of ethanol and water.[4] Ethidium has been shown to bind preferentially to the low salt form.[5] The first qualitative insight about the structure of poly(dG-dC)·poly(dG-dC) at high salt concentrations came from the work of Patel et al.[6] Their nuclear magnetic resonance (NMR) studies suggested that in these structures the symmetry unit repeats every two base pairs, the base pairing to be likely of the Watson-Crick type, and that every other glycosidic torsion angle and phosphodiester linkage adopts values other than those in B-DNA. Extrapolation of the results from single crystal crystallographic studies of hexamer[7] and tetramer duplexes[8,9] of dG-dC sequences, as well as data from fiber studies,[10] indicated that the polymer duplex in high salt solution may exist as a left-handed double helix. Recently this laboratory[11] carried out a very detailed computation of the magnetic shielding constants from the x, y, and z coordinates of the left-handed Z-DNA[7] and the right-handed Arnott and Hukins' B-DNA (herein after called A/H B-DNA) and compared them with the experimental NMR data for poly(dG-dC)·poly(dG-dC) in high salt solution. The computed shielding constants[11] for Z-DNA showed a remarkable agreement with the experimental data and that for A/H B-DNA showed significant deviations. This led to the conclusion that poly(dG-dC)·poly(dG-dC) in high salt solution can take up the Z configuration and does not assume the structure of A/H B-DNA. However it is vital to point out that this does not mean that Z-DNA is *the only structure* that poly(dG-dC)·poly(dG-dC) in high salt solution can assume. In order to reach this conclusion one has to carry out similar studies of other possible models and show that NMR can distinguish among these models and that the experimental data provide a unique fit to Z-DNA. For example, Olson[12] has advanced a right-handed vertical double helix in which the base planes are almost parallel to the helix axis as a model for the high salt form. Klug et al.[13] have advocated an alternating B-DNA model for alternating purine–pyrimidine se-

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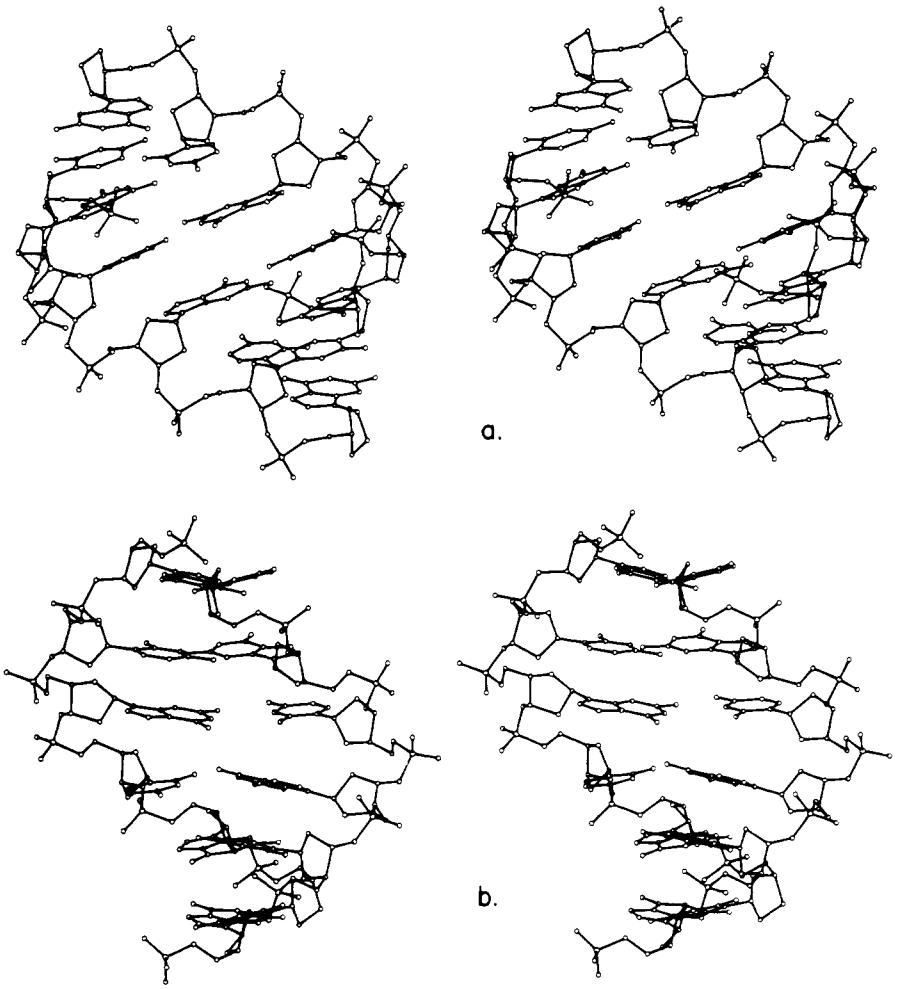

Figure 1. Stereographic perspective of A-DNA (a) and alternating B-DNA (b) for poly(dG-dC)·poly(dG-dC). Note that in Klug's alternating B model the base pairs are significantly propeller twisted. The authors[12] have never noticed this important nuance in their structure.

Table I. Torsion Angles in the Various Models[a]

model	backbone						sugar pucker	furanose					glycosyl torsion	χ
	ω'	φ'	ψ'	ψ	φ	ω		τ_0	τ_1	τ_2	τ_3	τ_4		
A-DNA[b]	314	178	83	46	208	275	^{3}E	4	334	37	324	21	anti	26
Alt-BDNA[c] 3'pG5'p	278	192	99	59	151	300	^{3}E	326	17	5	334	38	anti	33
Alt-BDNA[c] 3'pC5'p	227	200	143	65	172	293	^{2}E	326	42	327	15	12	anti	76
B-DNA[b]	265	155	156	36	214	313	$_3$E	356	25	325	33	342	anti	82
C-DNA[d]	254	161	156	37	200	321	$_3$E	356	25	325	33	341	anti	83
D-DNA[e]	259	142	156	69	208	298	$_3$E	356	24	325	33	341	anti	83
Olson's[f] B-DNA	268	198	86	58	178	295	^{3}E	0	340	30	329	20	high anti	122
Levitt-DNA[g]	273	170	108	68	168	294	^{04}E-^{1}E	319	36	357	338	39	anti	48
Z1-DNA[h] 3'pG5'p	291	256	99	190	179	48	^{3}E	8	340	24	340	7	syn	248
Z1-DNA[h] 3'pG5'p	80	266	138	56	221	223	^{2}E	334	35	330	17	6	anti	21
Z2-DNA[h] 3'-pG5'p	55	181	93	157	193	92	^{3}E	345	358	16	334	25	syn	241
Z2-DNA[h] 3'pC5'	74	259	147	66	163	146	$_3$E	336	39	322	26	359	anti	33

[a] All angles are in degrees. Note that $_3$E ≃ ^{2}E. [b] Reference 14. [c] Reference 13. [d] Reference 16. [e] Reference 15. [f] Reference 12. [g] Reference 17. [h] Reference 8.

quences; also, the D-DNA is supposed to be true for such sequences.

In this paper we attempt a comprehensive and systematic study of the NMR shielding patterns for CG sequences for the diverse spatial configuraitons of DNA whose x, y, and z coordinates are available. From a comparison of these theoretical shielding profiles with the experimental data and introducing proper modifications into the starting static structures, we synthesize the dynamic spatial configurations of DNA double helixes in solution.

To provide the background and to drive home the geometric differences among these diverse structures in Figures 1 through 5, we stereographically illustrate for poly(dG-dC)·(dG-dC) the structures of A-DNA, alternating B-DNA, A/H B-DNA, C-DNA, D-DNA, Olson's B-DNA, Z1/Z2-DNA, and Levitt and

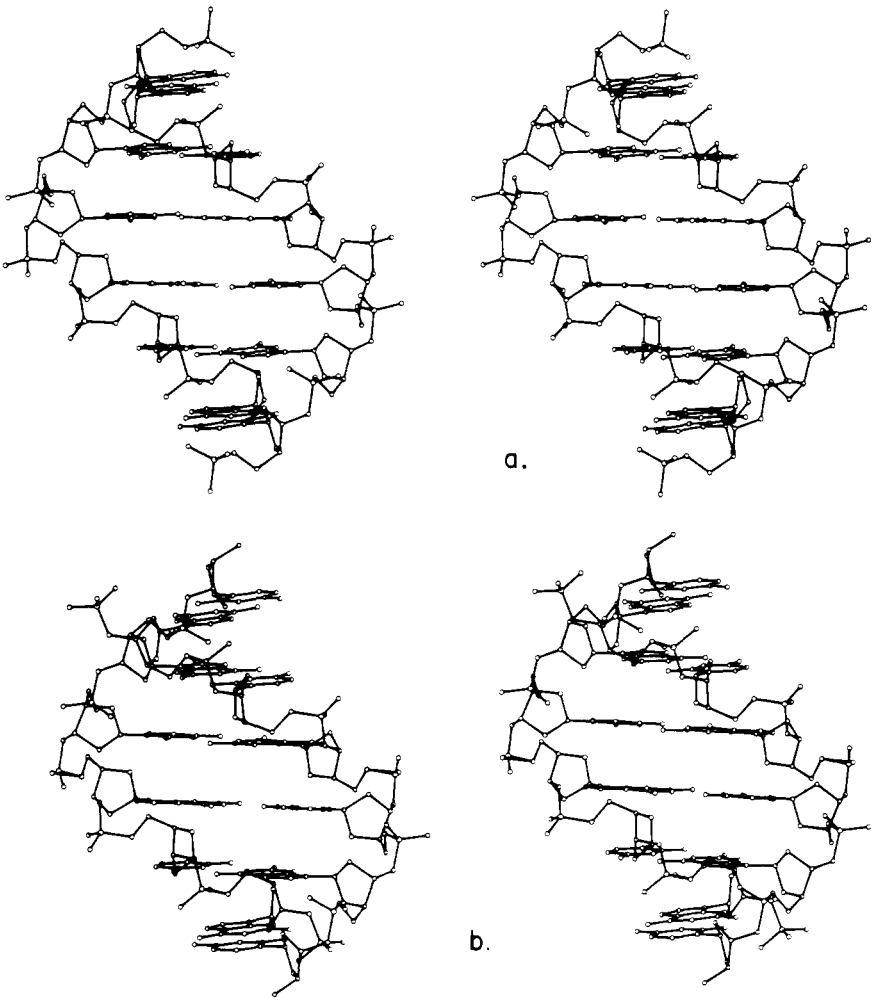

Figure 2. Stereographic perspective of Arnott and Hukins B-DNA (a) and C-DNA (b) for poly(dG-dC)·poly(dG-dC).

Dickerson propeller twist models.[17,18] In Table I, we have derived the torsion angles from the coordinates, and the nomenclature is explained in Figure 6. The x, y, and z coordinates for deriving the above torsion angles and for deriving the magnetic shielding constants were obtained from Arnott and co-workers[14,15] and Marvin et al.[16] for A, B, C, and D forms (Figure 1a, 2, and 3a). For the alternating B model (Figure 1b) it was taken from Klug et al.[13] Those for Olson's B-DNA (Figure 3b) were kindly provided by Olson, and those for Z1- and Z2-DNA (Figure 4) were kindly provided by Rich; those for Levitt (Figure 5a) and Dickerson (Figure 5b) were kindly provided by the authors. The only change we have introduced into some of these original coordinates was to substitute G for A and C for T. For example, the alternating B-DNA was originally proposed by Klug[13] for the poly(dA-dT)·poly(dA-dT) sequence. We modified the coordinates by replacing A with G and T with C (Figure 1b) mainly to investigate whether poly(dG-dC)·poly(dG-dC) can really access the alternating B-DNA structure in solution. In the Dickerson[18] model, we confined ourselves to the coordinates from the central GAATTC stretch of the self-complementary d-CGCGAATTCGCG double helix. This is because in the Dickerson[18] crystal structure it is this domain which displays significant propeller twists.

Methodology

The chemical shift of a given nuclei, for example, a proton, in the NMR spectrum is strongly influenced by the local geometric and chemical environment of the nuclei. An examination of Figures 1 through 5 clearly illustrates the significant geometric differences among the various helixes. Even though A-, B-, C-, D- and Olson's B-DNA's are made up of a monomer repeat unit, the A and Olson's forms have ^{3}E sugar pucker; the sugar pucker of B-, C-, and D-DNA lie in the ^{2}E domain. In the A form, the χ_{CN} is low anti and the bases are tilted about 19° to the helix axis; in the Olson's form, the χ_{CN} is high anti, the bases are almost parallel to the helix axis, and the structure has a vacant inner core of about 35 Å in diameter. The B, C, and D forms belong to a close family of structures, as an examination of the torsion angles in Table I reveals.

Even though the alternating B-DNA and Z1/Z2-DNA's have a repeat unit of a dinucleotide, the former generates a right-handed helix, but the latter generates left-handed helixes; in the former, the G is anti and in the latter it is syn. Furthermore, there are significant differences in the various torsion angles (Table I) between the alternating form and the Z forms, and these create morphological differences in the double helix (Figures 1b and 4). It should be noted that even though both alternating B- and Z-DNA's are antiparallel, in the Z forms at each base pair there is local parallelism because the sugar direction undergoes local inversion to accommodate a syn dG to enter into Watson–Crick base pairing in such a way that the glycosyl orientations are cis. A principal feature of the Levitt[17] model is the propeller twist (Figure 5) between the base pairs. In the Dickerson[18] model for the self-complementary d-CGCGAATTCGCG this twist can be seen clearly in the middle portion and becomes vanishingly small toward the ends of the helix (vide infra, Figure 9). An important feature of the Dickerson model is that it has no diad axis, and each of the 24 nucleotidyl units has a significantly different spatial configuration, so much so that the two complementary strands are not only nonequivalent but they are structurally revealingly different (Figure 5b).

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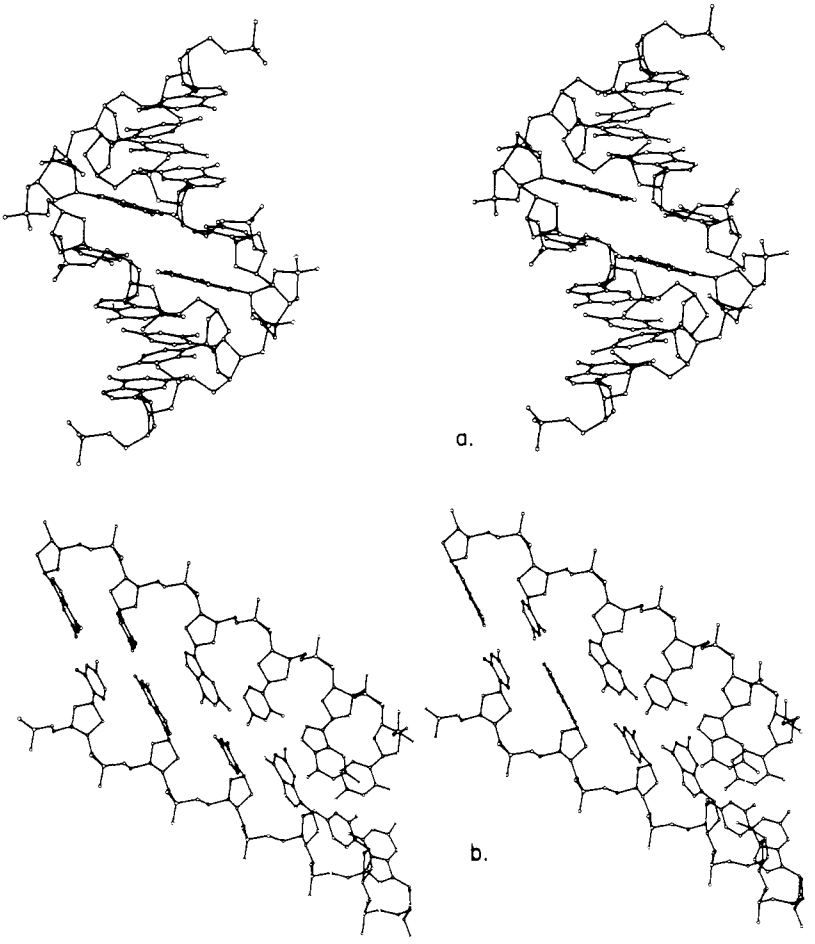

Figure 3. Stereographic perspective of D-DNA (a) and Olson's vertical double helix (b) for poly(dG-dC)·poly(dG-dC).

We have described elsewhere in extenso the assumptions[11] and principles involved in the computation of shifts, i.e., magnetic shielding constants, for a double helix. However, to make this presentation as self-contained as possible, we provide a brief summary here. We assume that the chemical shift of the central base-paired nucleotides in a heptamer duplex is the shift of protons

$$\begin{array}{l}\xrightarrow{\qquad\qquad}\\ \text{d-CGCGCGC}\\ \text{d-GCGCGCG}\\ \xleftarrow{\qquad\qquad}\end{array}$$

in any nucleotide unit in the polymer duplex and that there are no end effects. This assumption is reasonable because the polymer contains close to 100 base pairs and there is translational symmetry; the chemical shifts are not significantly affected by units beyond the third neighbor. We assume that the heptamer duplex can exist in any of the ten spatial configurations illustrated in Figures 1–5. In Figure 7 we have schematically drawn a heptamer segment. The chemical shifts of the central cytidine C_0 (Figure 7) will be affected by the remaining 13 nucleotide units.[11] Extensive calculations in this laboratory and that of Pullman and Giessner-Prettre[19] have shown that nucleotide units as far away as the sixth neighbor can affect the shifts. However, the chemical shifts are not significantly affected by nucleotide units beyond the third neighbor.

The contribution to the chemical shifts originate from (a) ring-current effect of the bases, (b) the diamagnetic component of the atomic magnetic anisotropy of the bases, and (c) the diamagnetic and paramagnetic components of the atomic magnetic anisotropy of the sugar–phosphate backbone. Shielding constants for a given proton of a nucleotide unit in structures like the ones in Figures 1–5 can be computed from x, y, and z coordinates taking all the above contributions into account.[11,20–22] *The calculated shielding constant essentially provides the magnitude and direction of shielding a proton in a nucleotide unit such as C_0 (Figure 7) will experience as the unit is moved from an isolated environment to that in an organized structure like the ones in figures 1–5.* In such a calculation one cannot include the contribution to shielding from the parent nucleotide unit to which the proton belongs; i.e., C_0 should be excluded when computing the effect on C_0 from the remaining 13 units in configurations Figures 1 through 5. G_0 should be excluded when computing the effect of the remaining 13 units on G_0. Here one is assuming that the conformation of the isolated mononucleotide is the same as it is in the various organized structures (Figures 1–5). This is not true; hence, a calculation at a second level should be undertaken to correct for the effect of this on shifts, and this is presented later.

The ring-current constants and the magnetic anisotropy tensor elements for the calculations were kindly provided by Pullman and Giessner-Prettre before publication.[23] Using the methods described elsewhere[11,20–22] and from the x, y, and z coordinates, we have calculated the magnetic shielding constants for CH5, CH6, CH1′, GH8, and GH1′ for the central C_0-G_0 base pair in a heptamer segment (Figure 7) of poly(dG-dC)·poly(dG-dC) for the ten different spatial configurations it may display (Figures 1–5). For these computations we have taken into account contributions to shielding from ring-current effects, as well as effects

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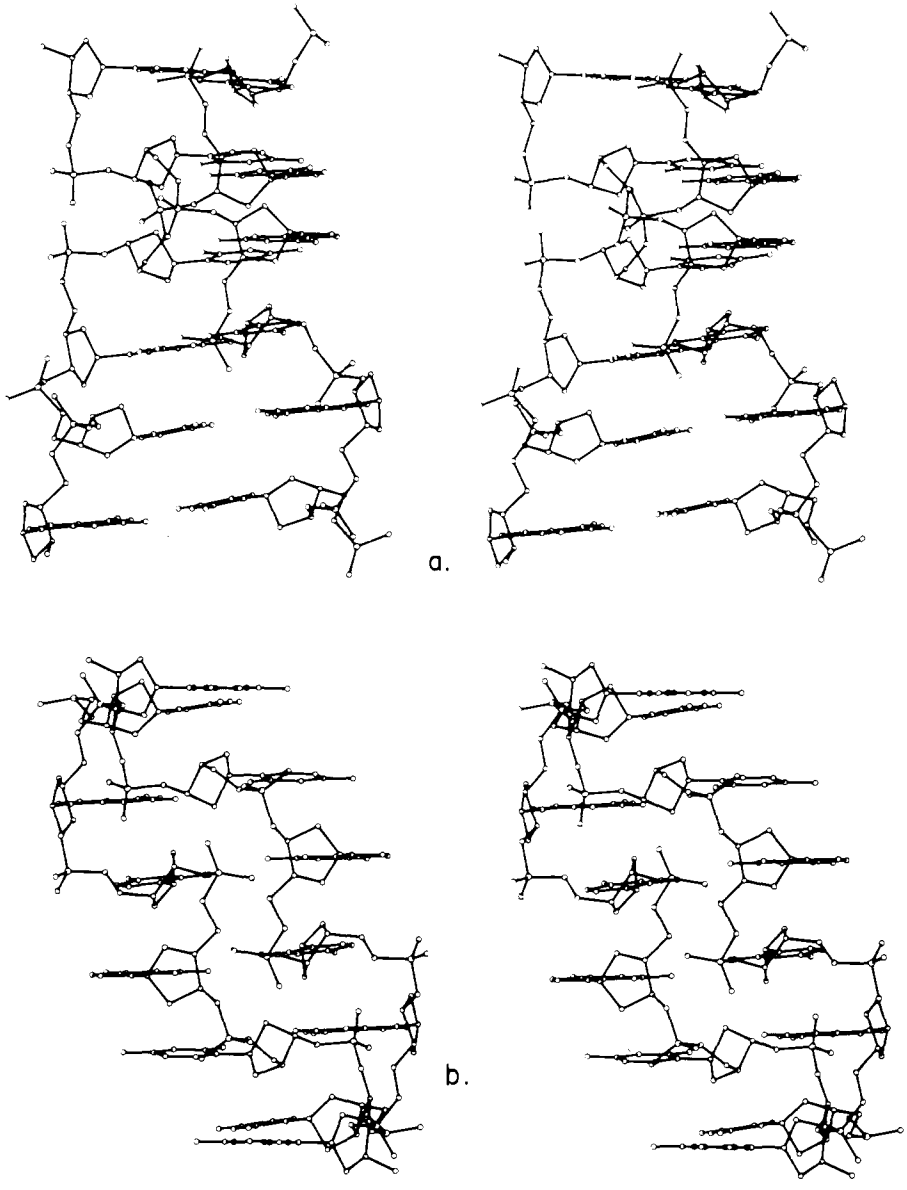

Figure 4. Stereographic perspective of Z1-DNA (a) and Z2-DNA (b) for poly(dG-dC)·poly(dG-dC).

from the diamagnetic and paramagnetic components of the atomic magnetic anisotropy. The results of the computations are presented in Table II. Before one examines Table II, we should point out that in the first paper in this series,[11] where we presented for the first time the NMR methodology to handle the details of a double helix, we have presented an extensive table which indicates the magnitudes of the various factors such as ring currents and the paramagnetic and diamagnetic component of the atomic anisotropy from various nucleotide units in a double helix. In Table II, the data are presented in a considerably reduced format and indicate only the contribution from the various neighbors. Thus, the vertical columns 1 through 7 respectively provide the contributions from (1) the complementary unit, (2) the total contribution from the two nearest neighbors on the same strand, (3) the total contributions from the two nearest neighbors on the complementary strand, (4) the total contribution from the two next nearest neighbors in the same strand, (5) the total contribution from the next nearest neighbors in the complementary strand, (6) the total contribution from the two next next nearest neighbors on the same strand, and (7) the total contribution from the two next next nearest neighbors on the complementary strand. The last column gives the total change in shift a given proton in a mononucleotide will undergo if it is to become part of any of these organized structures. For example, the last column indicates that CH5 will undergo a total shielding of 1.47 ppm if poly(dG-dC)·poly(dG-dC) were in the A form; out of this 1.47 ppm, –0.098 is contributed by the complementary G, 1.39 is contributed by the nearest neighbors G_1 and G_{-1}, and so on.

How does one experimentally measure the calculated shifts? One has to experimentally obtain the shifts for the poly(dG-dC)·poly(dG-dC) duplex in the solvent condition of interest and measure those for isolated mononucleotides, i.e., the same solvent conditions at extremely low concentration and high temperature. The difference in shifts between the experimental value for the monomers and the double helix should then be compared with those computed for the various spatial configurations. However, there is a problem. As you may recall, in these calculations the shielding effect of 13 neighboring nucleotide units arranged in a particular configuration on the shift of a nucleotide unit was carried out and we neglected, in this first level of calculation, the effect of its own local geometry on its shifts. This would have been alright if the experimentally measured isolated mononucleotides had a geometry identical with what they had in the organized structure. In fact, a plethora of NMR studies from this and other laboratoires[24-29] have shown that monomers, par-

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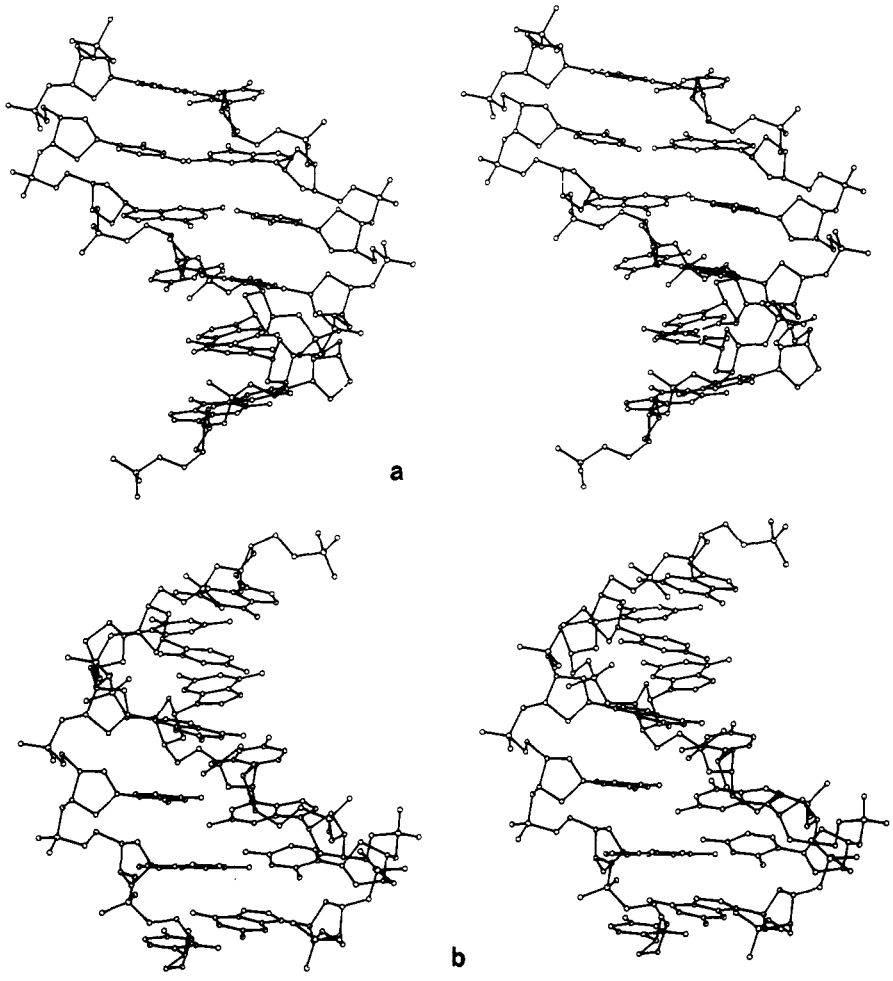

Figure 5. Levitt (a) and Dickerson (b) propeller twist models in stereo for poly(dG-dC)·poly(dG-dC).

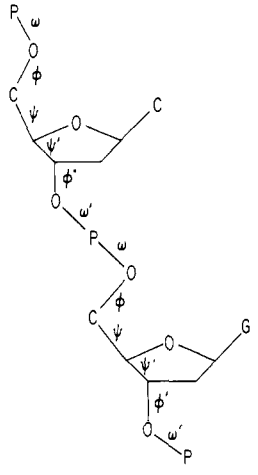

Figure 6. The nomenclature employed for the torsion angles for data in Table I. Note that for A, B, C, D, and Olson's DNA the repeat unit is a mononucleotide. For the alternating B and the Z1 and Z2 structures, the repeat unit is a dinucleotide. For these latter systems we have at first given the torsion angles for the pGp unit starting with ω' (P-03-) and we have reported the angles for the pCp unit starting with again ω'.

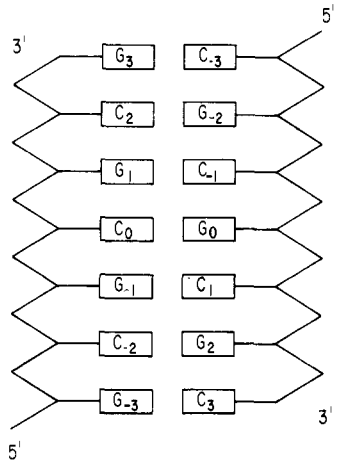

Figure 7. Schematic drawing of an heptamer segment of double stranded poly(dG-dC)·poly(dG-dC).

ticularly the purine ones, are highly flexible structure and that they are in a conformational blend. The shielding data in the last column of Table II do not take into account the change in shifts that will result as a conformationally equilibrating monomer is made part of an organized structure. Hence, a calculation at the second level should be made to correct the computed shielding values in Table II to reflect the true situation. The dominant factor which affects the shielding values of the observable protons is a change in χ as the monomer becomes part of the double helix. We have described in considerable detail[11] how calculation at the second level can be done and how corrections can be made using experimentally determined syn $\rightleftarrows$ anti and $^2E \rightleftarrows {}^3E$ equilibrium for the monomers[24-29] and from the dependence of shielding constants on χ[30,31] for B- and Z-DNA. Similar calculations were

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(28) D. B. Davies, *Prog. NMR Spectrosc.*, **12**, 135 (1978).
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Table II. Contributions to the Chemical Shifts for CH5, CH6, CH1′, GH8, and GH1′ for Various DNA Models[a]

proton	DNA model	1	2	3	4	5	6	7	total
CH5	A-DNA	−0.0973	1.3906	−0.0606	0.0936	0.0655	0.0521	0.0260	1.4701
	Alt-BDNA	−0.0607	0.3659	−0.0402	0.0611	0.0618	0.0529	0.0252	0.4210
	B-DNA	−0.0981	0.7249	−0.0405	0.0694	0.0609	0.0461	0.0329	0.7956
	C-DNA	−0.1033	0.5511	−0.0397	0.0584	0.0613	0.0422	0.0335	0.6035
	D-DNA	−0.1131	0.4174	−0.0316	0.0400	0.0700	0.0327	0.0310	0.4564
	O-DNA[b]	−0.1137	0.1988	−0.0474	−0.0155	0.0471	0.0064	0.0041	0.0798
	Levitt[c]	−0.0630	0.6133	−0.0424	0.0780	0.0349	0.0609	0.0264	0.7081
	Dickerson[b]	−0.0855	0.7546	−0.0460	0.0919	0.0282	0.0674	0.0275	0.8381
	Z1-DNA	−0.0932	0.7533	0.0727	0.0228	0.1361	0.0239	0.0229	0.9385
	Z2-DNA	−0.0169	0.7067	−0.0001	0.0189	0.0161	0.0213	0.0184	0.7544
CH6	A-DNA	−0.0695	0.4295	−0.0428	0.0353	0.0714	0.0239	0.0237	0.4715
	Alt-BDNA	−0.0536	0.2341	−0.0352	0.0514	0.0011	0.0407	0.0130	0.2515
	B-DNA	−0.0696	0.1040	−0.0383	0.0394	0.0389	0.0296	0.0290	0.1330
	C-DNA	−0.0724	0.1037	−0.0391	0.0409	0.0328	0.0311	0.0294	0.1264
	D-DNA	−0.0703	0.0779	−0.0371	0.0324	0.0209	0.0249	0.0268	0.0755
	O-DNA	−0.0770	0.6522	−0.0397	0.0050	0.1214	0.0005	0.0121	0.6715
	Levitt	−0.0509	0.2997	−0.0406	0.0552	0.0133	0.0448	0.0206	0.3421
	Dickerson	−0.0684	0.3267	−0.0398	0.0603	0.0107	0.0497	0.0212	0.3604
	Z1-DNA	−0.0705	0.5507	−0.0275	0.0561	0.0721	0.0263	0.0323	0.6395
	Z2-DNA	−0.0226	0.5235	0.0665	0.0473	0.0214	0.0243	0.0223	0.6227
CH1′	A-DNA	−0.0757	0.0980	−0.0181	0.0105	0.0980	0.0095	0.0185	0.1407
	Alt-BDNA	−0.0719	0.2683	−0.0373	0.0662	−0.0018	0.0389	0.0200	0.2824
	B-DNA	−0.0755	0.2573	−0.0343	0.0491	0.0542	0.0292	0.0270	0.3070
	C-DNA	−0.0761	0.4237	−0.0375	0.0639	0.0423	0.0382	0.0301	0.4846
	D-DNA	−0.0741	0.5592	−0.0438	0.0675	−0.0052	0.0402	0.0416	0.5854
	O-DNA	−0.0747	0.1539	0.0338	0.0042	0.0947	0.0001	0.0082	0.2118
	Levitt	−0.0917	0.2021	−0.0427	0.0385	0.0182	0.0357	0.0289	0.1890
	Dickerson	−0.0981	0.2505	−0.0295	0.0533	0.0252	0.0459	0.0298	0.2771
	Z1-DNA	−0.0731	0.3016	0.0374	0.1171	0.0451	0.0460	0.0413	0.5154
	Z2-DNA	−0.0538	0.3322	0.0739	0.1028	0.0612	0.0431	0.0318	0.5912
GH8	A-DNA	−0.0366	0.3710	−0.0569	0.1051	0.0199	0.0129	0.0452	0.4606
	Alt-BDNA	−0.0333	0.3410	−0.0634	0.1339	0.0006	0.0170	0.0292	0.4250
	B-DNA	−0.0355	0.1617	−0.0536	0.0730	0.0124	0.0135	0.0576	0.2291
	C-DNA	−0.0371	0.1661	−0.0552	0.0737	0.0141	0.0130	0.0556	0.2302
	D-DNA	−0.0359	0.1236	−0.0536	0.0584	0.0201	0.0077	0.0390	0.1593
	O-DNA	−0.0402	0.1540	0.0835	0.0168	0.0804	−0.0036	0.0116	0.3025
	Levitt	−0.0360	0.3386	−0.0547	0.1197	0.0073	0.0112	0.0356	0.4277
	Dickerson	−0.0349	0.3405	−0.0569	0.1242	0.0143	0.0141	0.0411	0.4424
	Z1-DNA	−0.0379	−0.0161	−0.0258	0.0159	0.0175	0.0130	0.0135	−0.0217
	Z2-DNA	−0.0023	−0.0073	−0.0156	0.0187	0.0040	0.0070	0.0181	0.0226
GH1′	A-DNA	−0.0396	0.0938	0.0301	0.0303	0.0458	0.0049	0.0352	0.2005
	Alt-BDNA	−0.0319	0.2255	−0.0811	0.0928	0.0112	0.0158	0.0417	0.2740
	B-DNA	−0.0397	0.3225	−0.0402	0.0680	0.0236	0.0146	0.0566	0.4054
	C-DNA	−0.0407	0.4692	−0.0588	0.0959	0.0203	0.0171	0.0634	0.5664
	D-DNA	−0.0413	0.6077	−0.0917	0.1129	0.0164	0.0145	0.0732	0.6917
	O-DNA	−0.0400	−0.0003	0.2360	0.0087	0.0582	−0.0019	0.0120	0.2727
	Levitt	−0.0430	0.2168	−0.1134	0.0987	0.0269	0.0129	0.0445	0.2304
	Dickerson	−0.0501	0.2708	−0.0484	0.1279	0.0471	0.0137	0.0539	0.4149
	Z1-DNA	−0.0294	0.0131	−0.0279	0.0261	0.0150	0.0231	0.0300	0.0238
	Z2-DNA	−0.0048	0.0010	−0.0173	0.0320	0.0074	0.0061	0.0241	0.0485

[a] See text for an explanation of the various column headings. Chemical shifts are in parts per million. [b] O-DNA = Olson's B-DNA. [c] Levitt propeller twist DNA. [d] Dickerson propeller twist DNA.

performed in the present case to the various forms of DNA studied. Last time[11] we did not carry out calculations at the second level for the shifts of CH5, CH6 and CH1′; however, we have done so in the present study. This was done on the basis that pyrimidine nucleotides overwhelmingly exist in the anti conformation[24-29] and the expected average value[33,34] of χ is $\sim 50°$. The final set of shielding constants obtained are given in Table III.

Chemical shifts of isolated mononucleotides in 10 mM and 4 M salt solutions were obtained by recording the ^{1}H NMR spectra of 3′dCMP, 5′dCMP, 3′dGMP, and 5′dGMP (8 mM, 90 °C) using the super-conducting 270-MHz FT NMR spectrometer. For all practical purposes, the shifts of the monomers were identical at 10 mM and 4 M salt concentrations at 90 °C at 8 mM concentrations of the monomer. The averages of the shifts for CH5, CH6, CH1′, GH8, and GH1′ for the 3′ and 5′ mononucleotides were taken. The shifts for the duplex of poly(dG-dC)·poly(dG-dC) in high salt solution were obtained from Patel.[35] For the low salt form, the data for poly(dG-dC)·poly(dG-dC), 10 mM in total nucleotides, were obtained in 3 mM NaCl buffered at neutral pH. The data were obtained at 81 °C. Such a high temperature was used because at lower temperatures the resonances were too broad to measure the shifts accurately. The onset of the melting of the duplex was at 89 °C, so much so that our structure in low salt solution corresponds to the form, a few degrees before melting. It is possible that our low salt temperature corresponds to the domain of high-energy transient base pair breakage and melting.[36,37] The differences in chemical shifts between the monomers

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(32) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **65**, 189 (1977).
(33) M. Sundaralingam, *Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 417 (1973).
(34) We have derived the torsion angles for the self-complementary d-CGCGAATTCGCG (**18**) from the x, y, and z coordinates. The average of the χ_{CN} of the six internal dC's is 51°. The χ_{CN} of the dG's vary from a low value of 60° to a high value of 93° in the double helix.
(35) D. J. Patel in "Stereodynamics of Molecular Systems", R. H. Sarma, Ed., Pergamon Press, New York, 1979, p 397.
(36) N. R. Kallenbach, C. Mandel, and S. W. Englander, in "Nucleic Acid Geometry and Dynamics, R. H. Sarma, Ed., Pergamon Press, New York, 1980, p 233.
(37) M. Nakanishi and M. Tsuboi, *J. Mol. Biol.*, **124**, 61 (1978).

Table III. Theoretically Computed Magnetic Shielding Constants and Those Experimentally Observed for Poly(dG-dC)·Poly(dG-dC) under High and Low Salt Conditions

proton	DNA model	computed shielding ($\Delta\delta$)	experimental $\Delta\delta$ high salt	experimental $\Delta\delta$ low salt
CH5	A-DNA	1.49		
	Alt-BDNA	0.46		
	B-DNA	0.85		
	C-DNA	0.65	1.05	0.82
	D-DNA	0.51		
	O-DNA[a]	0.24		
	Z1-DNA	0.95		
	Z2-DNA	0.75		
	Levitt[b]	0.71		
	Dickerson[c]	0.84		
CH6	A-DNA	0.20		
	Alt-BDNA	0.72		
	B-DNA	0.73		
	C-DNA	0.73	0.62	0.70
	D-DNA	0.68		
	O-DNA	1.68		
	Z1-DNA	0.51		
	Z2-DNA	0.45		
	Levitt	0.34		
	Dickerson	0.36		
CH1'	A-DNA	0.45		
	AH-BDNA	0.42		
	B-DNA	0.58		
	C-DNA	0.75	0.69	0.56
	D-DNA	0.86		
	O-DNA	1.15		
	Z1-DNA	0.91		
	Z2-DNA	0.77		
	Levitt	0.19		
	Dickerson	0.28		
GH8	A-DNA	−1.12		
	Alt-BDNA	−0.33		
	B-DNA	−0.15		
	C-DNA	−0.15		
	D-DNA	−0.22	0.30	0.27
	O-DNA	0.34		
	Z1-DNA	0.18		
	Z2-DNA	0.22		
	Levitt	−0.44		
	Dickerson	−0.80		
GH1'	A-DNA	−0.21		
	Alt-BDNA	−0.12		
	B-DNA	0.25		
	C-DNA	0.41		
	D-DNA	0.53	0.15	0.41
	O-DNA	0.42		
	Z1-DNA	0.17		
	Z2-DNA	0.20		
	Levitt	−0.35		
	Dickerson	−0.21		

[a] Olson's B-DNA. [b] Levitt propeller twist DNA. [c] Dickerson propeller twist DNA.

and the duplex poly(dG-dC)·poly(dG-dC) ($\Delta\delta$) under high and low salt conditions are listed in Table III. The assignments of the various resonances employed in the present study are identical with the ones employed previously.[11]

Spatial Configuration of Poly(dG-dC)·Poly(dG-dC) in High Salt Solution. A comparison of the computed shielding constants for the ten different spatial configurations of DNA with the experimental data for poly(dG-dC)·poly(dG-dC) at high salt concentration brings out a revealing story. In view of the large number of structures and the numbers to compare, we show in Figure 8 a computer-drawn histogram for effective visual comparison. We have indicated elsewhere[11] that in comparing computed shielding constants for a given structure with experimental data, one has to look for agreement individually for a collection of protons and that an agreement between theoretical computation and experimental data should be considered excellent if the difference is

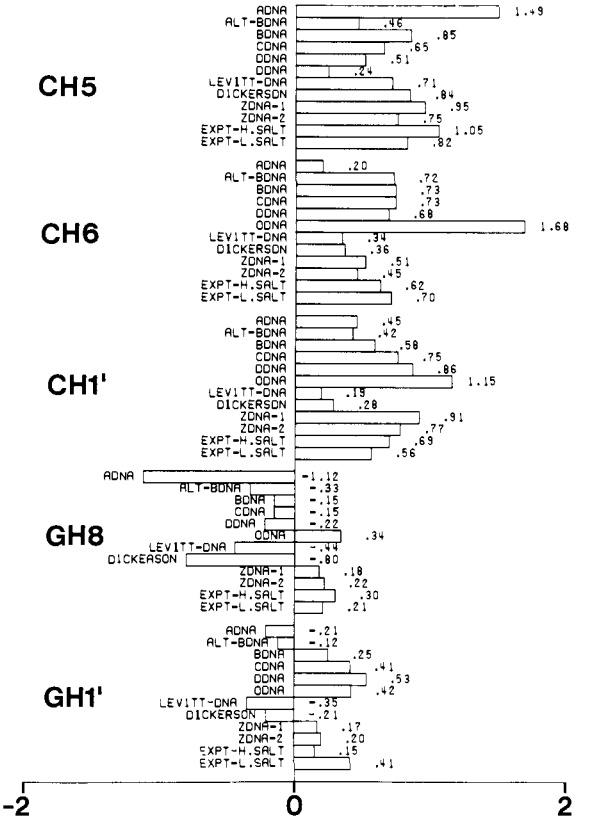

Figure 8. The histogram corresponding to Table III.

about 0.1 ppm and very good to fair if it is about 0.15 to 0.2 ppm. Any difference beyond 0.25 ppm is poor and unsatisfactory. Further, certain protons are more crucial than others. For example, in the present case CH5, whose shift is entirely dependent upon overall geometry and little affected by small internal local fluctuations, should show agreement within 0.1 ppm.

Examination of the data in Table III and the histogram in Figure 8 shows that out of the five separate sites (i.e., CH5, CH6, CH1′, GH8, and GH1′) we have used in the double helix, there is overall agreement between the computations and experimental observations in high salt solution in each case for Z1-DNA (Figure 4a); in four sites (CH5, CH6, GH8, and GH1′) the agreement is excellent (0.02 to 0.12 ppm), and in one case (CH1′) it is fair (0.22 ppm); it may be noted that the theory overestimates the shielding of CH1′ by 0.22 ppm. It is crucial to realize that a small fluctuation in the χ_{CN} of cytosine, which will cause a 3 to 4° increase in the average χ_{CN} in a solution of dC, of Z1-DNA (i.e., $\chi_{CN} \simeq 24$–25° instead of the presently used 21°) will practically compensate the above and will not affect significantly $\Delta\delta$ CH_6, and this will result in excellent agreement with experimentally observed data. This is because the shifts of CH6 and CH1′ are such that δ CH1′ is considerably sensitive and that δ CH6 is a little sensitive when χ_{CN} is increased from 20 toward 40°.

In the case of Z2-DNA (Figure 4b), the data start with a disagreement of 0.3 ppm between the calculated and experimentally observed values for CH5. This is a serious disagreement because the shift of CH5 is independent of local fluctuations and suggests that Z2-DNA may be an untenable structure for poly(dG-dC)·poly(dG-dC) in high salt solution. It is true that for the Z2 form one notices excellent agreement for GH8 and GH1′, but this is most likely accidental because both Z1 and Z2 forms predict the same magnitude for GH8 and GH1′. Particularly noteworthy is that the projected values for Z2 overestimate the shielding of CH1′ only by 0.08 ppm but underestimates the same for CH6 by as much as 0.17 ppm. The magnitude and direction of these shifts are such that they cannot be internally compensated by few degrees of fluctuations about the employed value of 33° for χ_{CN} of dC in Z2.

The vertical double helix was advocated by Olson[12] as a model for the high salt form of poly(dG-dC)·poly(dG-dC) from theo-

retical considerations. Though this is an opulent and a rich piece of biological architecture (Figures 3b; see Dhingra and Sarma[38] for a breathtaking space-filling color stereograph of the vertical double helix), the data in Table III and Figure 8 resoundingly show that this is totally an untenable structure for poly(dG-dC)·poly(dG-dC) in high salt solution. The data show that the experimental $\Delta\delta$ values are off by −0.81, 1.06, 0.46, and 0.27 ppm for CH5, CH6, CH1′, and GH1′ from the projected ones. The overwhelming underestimation of the shielding of CH5 is due to insufficient overlap and stacking among the bases in a given strand; the large overestimation of the shielding of CH6 and CH1′ is partly due to the high χ value of 122°, a situation in which CH6 has moved away from the magnetic anisotropy of the sugar ring oxygen and CH1′ has moved away from the same of >C=O at C2 of the heterocycle.

Even though in the alternating B-DNA[13] (Figures 1b) the repeat unit is a dinucleotide like Z1-DNA, the data (Table III, Figure 8) suggest that this cannot be the true structure for poly(dG-dC)·poly(dG-dC) in high salt solution. The structure predicts a shielding of CH5 by 0.46 ppm, whereas the observed value is 1.05 ppm. Furthermore, the structure overestimates the shielding of CH6 by 0.1 ppm and underestimates the shielding of CH1′ by 0.27 ppm. Local fluctuations of a few degrees around the employed χ_{CN} of 76° for dC cannot internally compensate these opposite trends for CH6 and CH1′. Also notice that there is a discrepancy of −0.63 ppm for GH8 and −0.27 ppm for GH1′ between what is projected by the structure and what is observed.

Arguments similar to the ones above (using the data in Table III, Figure 8) can be used to demonstrate that the classical structures like the A, B, C, and D forms or the new ones like the Levitt and Dickerson propeller twist forms do not hold true for poly(dG-dC)·poly(dG-dC) in high salt solution.

From the above discussion it is obvious that among the ten different spatial configurations for DNA examined, i.e., A, B, C, and D forms of DNA, the vertical double helix, the alternating B-DNA, the Z1- and Z2-DNAs, and the two propeller models, only the left-handed Z1-DNA correctly predicts the observed experimental data. It seems safe to conclude that Z1-DNA is most likely the only spatial configuration that poly(dG-dC)·poly(dG-dC) in high salt solution may assume. The time averaged, fluctuation averaged solution structure is essentially identical with the solid-state structure except that in the solution state, fluctuations about the χ_{CN} of dC may displace this χ_{CN} to an average value of 24–25° rather than the 21° projected from the single crystal studies of the self-complementary d-CGCGCG hexamer. It is crucial to realize that an average of 25° *does not* mean that their extreme values could be anything provided the average is 25°. This is because the shifts are not linearly dependent on the magnitude of torsion. For example, if the χ of dC changes from 20 to 30°, δ CH6 will be unaffected, but if it goes from 20 to 10°, δ CH6 will experience a high-field shift of 0.4 to 0.3 ppm! Because the conclusions are arrived at by examining the shift patterns of a fair number of protons and because the direction and magnitudes of the shifts are nonlinearly sensitive to torsional events, it is an inescapable conclusion that in those instances when we derive a single average solution structure, for example, Z1-DNA for poly(dG-dC)·poly(dG-dC) in high salt solution, it is a real structure with finite lifetime—it is not an average of some widely different spatial configurations. It is necessary to point out that, due to the lack of the availability of refined coordinates, we have been unable to examine the S-DNA of Arnott et al.[10]

Solution Spatial Configuration of Poly(dG-dC)·Poly(dG-dC) in Low Salt Solution. Examination of the projected magnetic shielding constants for the various forms of DNA vis-a-vis the experimentally observed $\Delta\delta$ for poly(dG-dC)·poly(dG-dC) in low salt solution (Table III, Figure 8) provides insights regarding its structure under low salt conditions. Such comparison, as was done before for the high salt form (where heavy weight was placed for the shift of CH5, the internal compensation between the shifts CH6 and CH1′ due to fluctuations about the χ_{CN} of dC was taken into account and overall agreement for a collection of protons were sought), clearly shows that A-DNA, D-DNA, the alternating B-DNA, the vertical double helix, the Z1- and Z2-DNA's or the propeller twisted forms of Levitt or Dickerson cannot be the true structures of poly(dG-dC)·poly(dG-dC) in low salt solutions.

In the case of the classic Arnott and Hukins[14] B-DNA, there is excellent agreement between projections and what is experimentally observed at three sites, i.e., CH5, CH6, and CH1′; in the case of GH1′, the agreement is good to fair, i.e., within 0.17 ppm, but GH8 shows a violent disagreement of up to 0.36 ppm. If one takes C-DNA, the situation is somewhat the same: the agreement for CH5 and CH1′ is less satisfactory than that for B-DNA, but the agreement that for GH1′ and CH6 is excellent. However, one should place high weight on the CH5 shift and should be able to explain discrepancy in CH1′ and CH6 due to fluctuations in the χ_{CN} of dC. Under these operational criteria, C-DNA fails even though we realize that the torsion angles for the AH/B- and C forms are close (Table I), and in solution internal fluctuations may allow accessibility to both forms. It is our thesis that the time-averaged, fluctuation-averaged structure lies close to the AH/B form and that the large discrepancy in GH8 shifts represent a special dynamical situation which the time-averaged and space-averaged fiber structure does not recognize, i.e., the ability of guanine to assume a syn conformation under our experimental conditions, i.e., at 81 °C, 8 °C below the onset of melting. It is possible that for very high temperature melting polymer duplexes, this temperature range may represent the high-energy breathing mode in which there is transient base pair opening and some very small percentage of bubble formation.

Our observation that as we increase the temperature from 70 to 81 °C there is detectable change in the line width suggests the onset of detectable motion or high-energy breathing at about 81 °C under our experimental conditions. Our data can be rationalized on the basis of an equilibrium between large populations of AH/B-DNA and very small populations of a model in which the dG is free to adopt the syn conformation. We are unable to obtain any detailed information about the small population of the syn form. It could originate from a bubble in which the dG is syn or it could be the unwound syn helix of Arnott.[46,47]

Examination of the dependence of δ CH8 in purine nucleotides[30,31] clearly reveals that a change of χ from 30 to 240° can cause an upfield shift of CH8 by as much as 1.5 ppm; i.e., even a very small percentage of the bubble in which the χ for dG is free to move from anti to syn range can explain our data. One cannot rationalize the data on the basis of an equilibrium between AH/B-DNA and Z1-DNA, unless one is willing to assume significant populations of the latter. There are several lines of independent evidence which can be "stretched" to support our thesis. Examination of the recently solved[18] crystal structure of the self-complementary d-CGCGAATTCGCG shows that the χ_{CN} for dG varies from as low as 60° to as high as 93° in the double helix;[34] in the crystal structure of double-stranded d-CGCG and d-CGCGCG, the dG in syn.[7-9] These suggest that even in the organized double helix the χ_{CN} about dG can vary considerably; extensive solution studies of mononucleotides and single-stranded oligonucleotides which are akin to bubbles have clearly shown[28] that, unlike the pyrimidine systems, the χ_{CN} of purine systems show considerable flexibility, so much so that they exist in a syn $\rightleftarrows$ anti equilibrium.

Are the G·C Pairs Propeller Twisted? Recently, Crothers and co-workers[39] have concluded that the base pairs in low salt poly(dG-dC)·poly(dG-dC) are not flat but propeller twisted and that the twist is smaller than what they reported for calf thymus DNA.[40] This does not at least prima facie agree with our conclusion that the low salt form of poly(dG-dC)·poly(dG-dC) is the

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(39) H. M. Wu, N. Dattagupta, and D. M. Crothers, *Proc. Natl. Acad. Sci. U.S.A.*, in press. Private communication to R.H.S. from D.M.C.

(40) M. Hogan, N. Dattagupta, and D. M. Crothers, *Biochemistry*, **18**, 280 (1979), and private communications to R.H.S. from D.M.C.

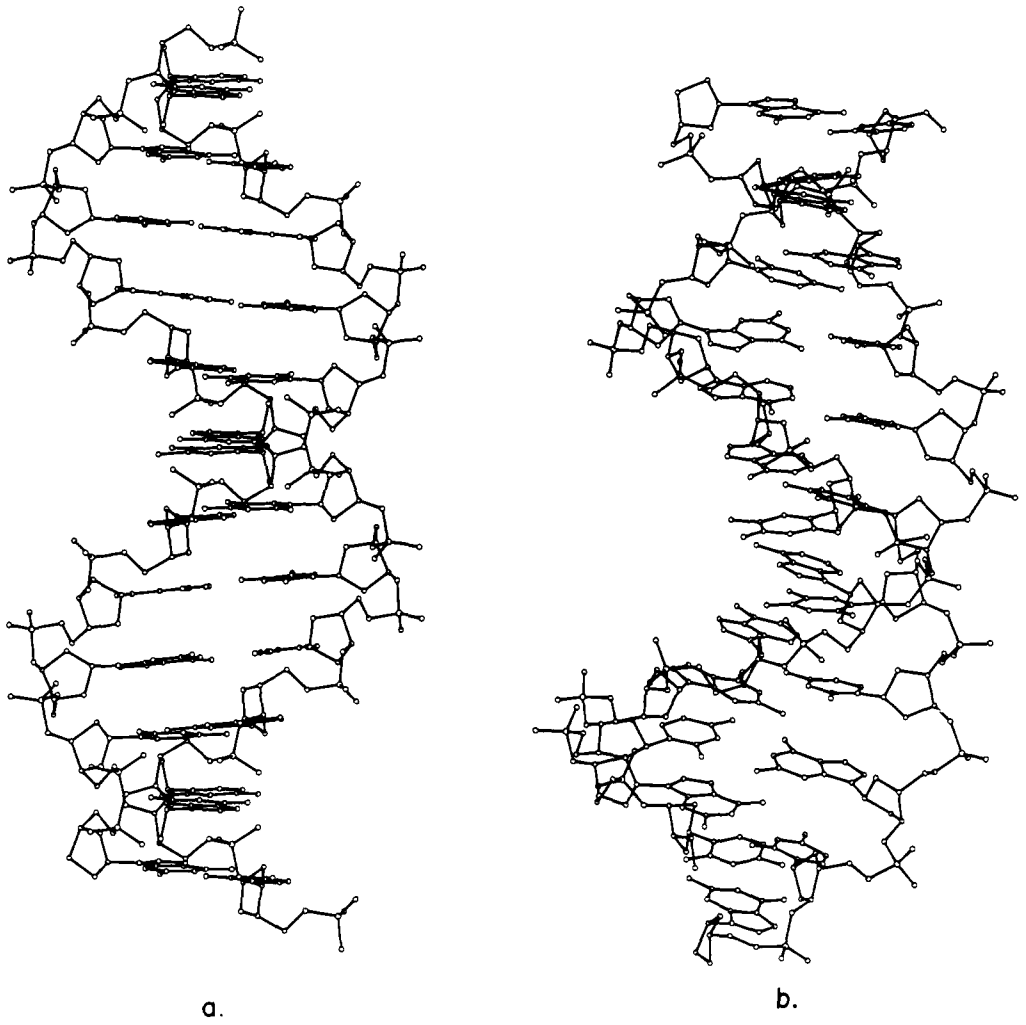

Figure 9. Spatial configuration of the self-complementary d-CGCGAATTCGCG in Arnott–Hukins B (a) and Dickerson's propeller twist models (b).

classic Arnott and Hukins' B-DNA in which the bases are flat. Before we present our explanations for these two apparently different findings from two separate spectroscopic methods, we want to examine available information in the literature.

Examination of the crystal structure of the self-complementary d-CGCGAATTCGCG[18] clearly illustrates that the AATT region is prominantly propeller twisted and that as one moves toward the ends in the GCG and CGC regions, propeller twists (Figure 9) becomes very small. The crystal structure of the miniature double-helix GpC clearly shows[41,42] that the bases are flat. These observations, along with the fact that there are three H bonds in GC pairs, suggest that GC pairs may have an intrinsic tendency to be flat.

However, Crothers and co-workers do not see a pronounced propeller twist in the GC systems.[39] The data in Table III make it vividly clear that Levitt and Dickerson propeller twist models are totally untenable. Comparison of the computed magnetic shielding constants with experimental data for the low salt form shows that among the *five* sites examined, only in *one* do these propeller models agree. For example, for CH6, Levitt and Dickerson models predict shielding of 0.35 ppm, whereas what is observed is 0.70 ppm; at CH1′, the models predict 0.19 and 0.28 ppm, respectively, but what is observed is 0.56 ppm. In the case of GH8 and GH1′, these models are off by 0.6 to over 1 ppm. There exists no internal motional mechanism in DNA to correct for these outrageous discrepancies. Obviously the Levitt and Dickerson propeller twist models are untenable for poly(dG-dC)·poly(dG-dC) in low salt solution.

Poly(dG-dC)·poly(dG-dC) in low salt solution can essentially maintain an organized structure of AH/B-DNA and could have a small twist between base pairs of $\simeq 10°$, distributed +5° for G and −5° for C (or −5° for G and +5° for C) from the AH/B-DNA base plane, and there could be interconversion, i.e., (+5°)G-C(−5°) $\rightleftarrows$ (−5°)G-C(+5°). In the NMR, especially at the employed 81 °C, it will appear as flat base pairs. It is possible that electric dichroism[39] is able to pick up such nuances and subtleties to which NMR with its long time scale is transparent. Obviously the total twist should be in the neighborhood of 10° and should be symmetrically distributed between the base pairs from the plane of AH/B-DNA. If it is larger, the nonlinear and directional dependence of shift vis-a-vis twist will reflect in the NMR data.

Plasticity of the DNA Double Helix. It is exciting to note that a DNA of dG-dC·dG-dC sequence can take up a left-handed Z1-DNA spatial configuration in one set of conditions, and in another set of conditions it can undergo so much local structural alterations that the very morphology and handedness display dramatic changes—a change from a thinner helix devoid of major grooves to a thicker helix with distinct major and minor grooves, the AH/B-DNA. Recently, Patel, Sarma,[43] and their co-workers

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(42) B. Hingerty, E. Subramanian, S. D. Stillman, T. Sato, S. B. Broyde, and R. Langridge, *Acta Crystallogr., Sect. B*, **32**, 2998 (1976).
(43) B. J. Wagner, C. K. Mitra, M. H. Sarma, R. H. Sarma, and D. J. Patel, submitted to *Biochemistry*.

have observed that in solution the AATT stretches of self-complementary d-CGCGAATTCGCG assumes pronounced propeller twists a la Levitt, Crothers, and Dickerson[17,18,39,40] and that in this system the AATT induces the nearest-neighbor GC's to assume propeller shapes.

Sarma, Ikehara, and their co-workers[48] recently demonstrated that changing the χ_{CN} from 80 to 120° in a double helix changes the handedness from right to left. It has been revealed by Rich and co-workers, discussed by Sarma et al.,[49] as well as by Felsenfeld and co-workers,[50] that 7-methylation of guanosine or 5-methylation of cytosine with a touch of Mg^{2+} promotes the transformation of the double helix from a right-handed to a left-handed structure. In a computer experiment, Olson[51] has shown that such transformations can be achieved by a torsional itinerary involving an all-trans backbone arrangement.[51] These results clearly suggest the rich plasticity in the structure of the DNA double helix and its ability to assume sequence and ionic strength dependent distinct spatial configuration. This vivid demonstration of the plasticity denotes the necessity of paying serious attention to the concept of long-range allosteric transformations, propounded by Crothers[44,45] as a mechanism for the control of genomic expression.

Possible Source of Errors and Their Effect on Our Conclusions. (a) The experimentally observed *total average change* in shifts from low salt to high salt for poly(dG-dC)·poly(dG-dC) is only 0.15 ppm; prima facie one may conclude that NMR is not sensitive to profound structural changes. This appears so only because one is *summing up the changes in each of the five sites and then dividing by five.* However, it should be noted that under favorable circumstances (vide infra), shifts are very sensitive to local structural changes and one should compare observed shifts for *each site* with those computed for *each site* (rather than averaging) and determine, among the various sites examined, how many of them agree and how many do not. If one examines a fairly large number of possible models (in the present case, 10) and a reasonable number of sites (in the present case, 5) and finds that only one model shows close agreement with *all the sites* examined, one can reach a conclusion with reasonable confidence that the specific model which shows agreement is probably correct. For example, in the present case we conclude that the structure of poly(dG-dC)·poly(dG-dC) in high salt form is most likely the Z1 form. The confidence level of such a conclusion increases if one can find independent data. For example, ^{31}P NMR studies of Patel[52] showed that in high salt poly(dG-dC)·poly(dG-dC), the two ^{31}P resonances are chemically shifted; NOE studies indicated[53] that the dG is syn in high salt. These independent studies, even though they do not in any way establish the structure, at least are consistent with the Z1 form.

(b) There could be profound local structural changes and it may not reflect in the NMR parameters because of accidental coincidences of shifts. An observed equivalence of shifts does not necessarily mean that the local structural details are the same or different. For example, it is disappointing to note that the observed δ CH8 of guanine of the polymer in the high and low salt form is the same. This very observation indicates the necessity of examining *as many separate sites* as possible and one should not average them. In the present case, we not only find agreement with what is calculated for the δ CH8 guanine of Z1-DNA, but also that for GH1′, CH1′, CH6, and CH5.

(c) Our conclusions are based on the belief that what we know about the conformations of the reference states (i.e., mononucleotides) in solution is correct. We have used data for these from publications from this laboratory[24-26] and from that of Guschlbauer and co-workers.[27] So far, the conformations that are proposed for the monomers in these papers[24-27] have been generally accepted to be true and the only area that needs rechecking is the syn/anti distribution.[27]

(d) Obviously, we cannot dismiss, once and for all, the possibility that in solution, the actual structure may be very different from the 10 models that are tested here. It is entirely possible that the NMR data may be compatible with a totally different structure—in fact, a fairly large number of totally different structures. No laboratory has the computer time required to search the entire 360° conformation space of each of the single bonds in single-stranded nucleic acids to generate a double helix whose theoretical chemical shift will agree with what is experimentally observed. In fact, such an exercise will be fruitless because in the present case one is dealing with just five sites which are experimentally accessible, a situation which does not permit what is called "unique fitting" when everything is floated. If one has available at least 2 dozen sites and several coupling constants, a unique fitting can be attempted a la Evans and Sarma[55] and Sarma and co-workers.[56] The problem lies in the inability of NMR, not withstanding the new generation of machines, to resolve lines for a slightly stiff polymer and the unfortunate chemistry of DNA that as lines begin to resolve at high temperatures, it begins to melt and change from the double helix to the random coiled single strands. The mission of the present article is a limited one. We have attempted to determine which structures, among the various structures proposed for the double helix from theory, fiber, and single crystal studies, are probably true in solution. We conclude for the high salt form of dG-dC sequences that it is the left-handed Z1 form; for the low salt form, we present data that suggest it to be the right-handed Arnott-Hukins B form in equilibrium with a small population of a form in which dG is syn. The recent discovery that the thousands of reflections from the single crystals of 5-I-d-CCGG are in uncanny agreement with what is expected from the A form, proposed more than a decade ago from the handful of fiber spots by Arnott and Hukins, makes one wonder whether there are strucures for the double helix beyond the conformational spaces occupied by the A, B, and Z families, other than variations within each family.

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