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Left-handed helices for DNA: Studies on poly[d(I-C)]

(conformational flexibility/stereochemical guidelines/duality in handedness/polymorphism of DNA)

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ABSTRACT **Earlier, we showed that, for the D form ($n = 8$ and $h = 3.03$ Å, where n is number of nucleotide units per turn and h is height per nucleotide unit) of poly[d(A-T)], both right- and left-handed double helical models are stereochemically satisfactory and give good agreement with the observed fiber diffraction data. It was also noted that the conformations of the right- and left-handed D-DNA models are very similar to those of the right- and left-handed B-DNA models. This observation was consistent with the D → B transition in the solid phase. As a continuation of our earlier studies, we have carried out similar experiments with poly[d(I-C)]. We could obtain a crystalline D-form pattern ($n = 8$, $h = 3.13$ Å) of the fiber at 75% relative humidity (r.h.); the hydrated (r.h. ≈ 95%) form of the same fiber gave the classical B-form pattern ($n = 10$, $h = 3.40$ Å). In the present report, we show that both right- and left-handed double-helical models are consistent with the fiber diffraction data of poly[d(I-C)] in the D-form. Theoretical energy calculations also suggest that the right- and left-handed B- and D-DNA models are almost equally stable. Hence, we conclude that the right- and left-handed double-helical models of poly[d(I-C)] in a given form (B or D) are equally likely and that the fiber diffraction data do not permit discrimination.**

A left-handed double helix was suggested by Mitsui *et al.* (1) as a possible model to explain the semicrystalline fiber pattern of poly[d(I-C)] in the D form ($n = 8$ and $h = 3.10$ Å). But the structure was found to be stereochemically unsatisfactory and hence was rejected by Arnott *et al.* (2). Instead, they proposed a right-handed double-helical model for the D form of DNA as observed for poly[d(A-T)] ($n = 8$, $h = 3.03$ Å). Further, it was found that the wet fibers of poly[d(A-T)] gave the classical B-form pattern ($n = 10$, $h = 3.40$ Å) as observed for a wide variety of natural DNAs (3). Since B-DNA was believed to be right-handed, the dimorphic D → B transition was taken to indicate that D-DNA should also be right-handed. However, on stereochemical grounds alone, the right-handed B- and D-DNA models (2, 4) of Arnott and co-workers are unacceptable (5, 6). This prompted us to reexamine the structures of B- and D-DNA. Earlier, we showed that both right- and left-handed duplexes are stereochemically possible for the B and D forms of DNA and that they give good agreement with the published intensity data (5, 6). Here, we report structural studies on the crystalline D form as exhibited by the Na salt of poly[d(I-C)] fibers at 75% relative humidity (r.h.) and reaffirm that stereochemically allowed right- and left-handed duplexes show quantitative agreement with the observed data.

Crystalline D form of poly[d(I-C)] and the dimorphic B → D transition in the solid phase

The D-form pattern of poly[d(I-C)] obtained by using a flat-plate camera is shown in Fig. 1*a*. The pattern is superior to those obtained earlier for the same specimen and for poly[d(A-T)]; 69 measurable reflections were indexed on the basis of a tetragonal cell with $a = 18.42$ Å and $c = 25.07$ Å. The fiber was tilted by about 7° such that the higher layer lines ($l \geq 7$) cut the sphere of reflection. Meridional reflections appeared to occur on seventh, eighth, and ninth layer lines. To resolve which was the true meridian, a precession photograph was recorded for the same specimen (Na salt of poly[d(I-C)]) under the same conditions (r.h., 75%). As shown in Fig. 1*b*, the precession photograph had a meridional reflection on the eighth layer line. This indicated that poly[d(I-C)] in the D form was an 8-fold ($n = 8$) helix with $h = 3.13$ Å. The size of the unit cell was such that only one 8-fold helix could pass through it. The intensity data of 69 spots were obtained from the peak heights of microdensitometer traces on each layer line; the intensities, so collected, were subsequently corrected for Lorentz and polarization effects (7).

The B-form pattern was obtained from wet fibers of poly[d(I-C)] (r.h., 95%). However, no quantitative analysis of the B-form was carried out because the pattern was of a semicrystalline nature (Fig. 1*c*).

Right- and left-handed models for poly[d(I-C)]

The values of the helical parameter h of poly[d(A-T)] and poly[d(I-C)] in the D form are only marginally different. For poly[d(A-T)], it was earlier shown that both right- and left-handed helical D-DNA models having the C2′-*endo*, tg^- conformation are possible (5, 6). It was observed that, in the same domain—i.e., C2′-*endo*, tg^-—both right- and left-handed helical models could be obtained that were consistent with the helical parameters and the intensity data of poly[d(I-C)] in the D form. The conformational parameters of the right- and left-handed helical models are given in Table 1. Both models involve the preferred correlation between the sugar pucker and the P—O torsions; i.e., the P—O torsions are tg^- for the C2′-*endo* pucker. It is interesting to recall that Mitsui *et al.* (1) chose an unusual 01′-*endo* puckering of the sugar residues for the left-handed model of poly[d(I-C)]. However, the model also had other steric strains and it was rejected. Although the right-handed D-DNA model of Arnott *et al.* (2) had the sugar pucker in the most favored C2′-*endo* region, the P—O torsions were g^-g^- instead of tg^-. The inevitable consequence was a low value of α (143°), which gave rise to serious steric compression between one of the pendant oxygens attached to the P and the C2′ atom of the sugar residue. The best right-handed B-DNA model of Arnott and Hukins (4) also had an unorthodox (C2′-*endo*,

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Abbreviation: r.h., relative humidity.

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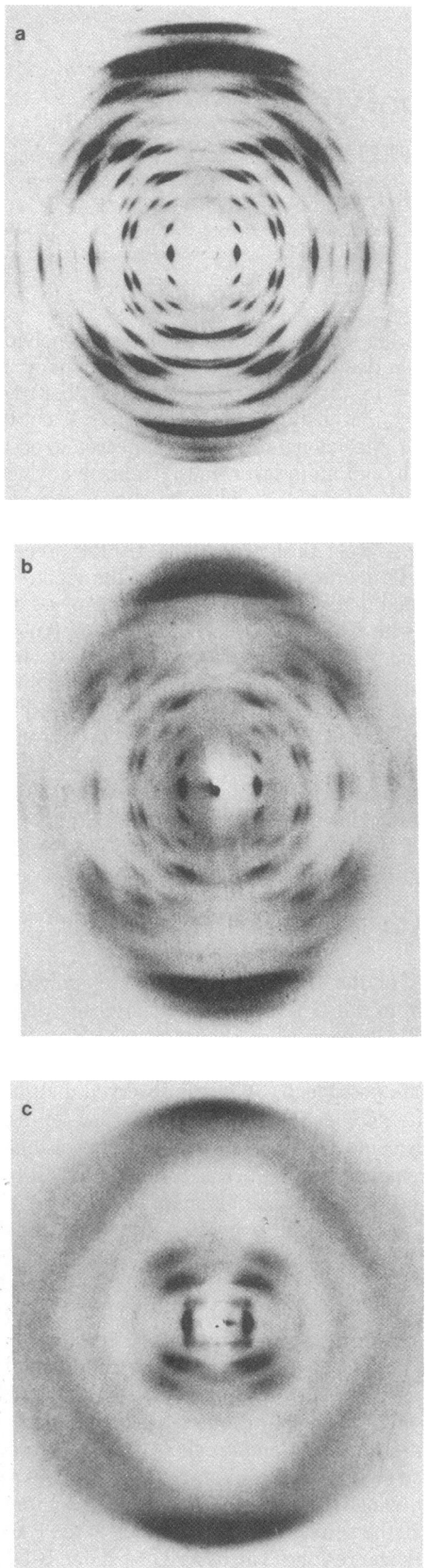

FIG. 1. X-ray diffraction patterns of the Na salt of poly[d(I-C)] fibers. (*a*) D-form pattern of poly[d(I-C)] fiber at 75% r.h. recorded on a flat-plate camera. Specimen-to-film distance, 5 cm. (*b*) Precession photograph of the poly[d(I-C)] fiber at 75% r.h. Specimen-to-film distance, 6 cm. (*c*) B-form pattern of wet fiber poly[d(I-C)] (r.h., 95%). The photograph was recorded on a flat-plate camera as in *a*.

Table 1. Conformational parameters and energy values of B- and D-DNA models of poly[d(I-C)]

	B DNA		D DNA	
	Right	Left	Right	Left
Backbone torsion angles, °				
α (C3′-O3′)	225	241	203	211
β (O3′-P)	208	204	218	213
γ (P-O5′)	313	270	308	267
δ (O5′-C5′)	141	135	148	163
ε (C5′-C4′)	40	37	58	34
ζ (C4′-C3′)	141	136	147	153
Glycosyl torsion, °				
χ [C1′-N9 (inosine)]				
χ [C1′-N1 (cytosine)]	69	−3	58	9
Furanose conformational angle, °				
σ_0 (C3′-C2′)	−36.7	−39.3	−34.4	−34.6
σ_1 (C4′-C3′)	18.7	18.9	22.6	26.1
σ_2 (O1′-C4′)	8.0	10.6	−1.7	−6.4
σ_3 (O1′-C1′)	−31.4	−35.9	−20.3	−16.5
σ_4 (C2′-C1′)	41.0	45.4	34.2	32.8
Base parameter*				
Tilt (θ_x), °	−5.0	−3.0	−25.0	2.0
Twist (θ_y), °	−4.0	0.0	0.0	0.0
Displacement (D), Å	−0.6	−1.2	−2.5	−3.0
Energy value†				
I-C sequence	−63.8	−56.8	−67.8	−61.4
C-I sequence	−64.8	−69.3	−61.8	−63.4
I-C-I sequence	−130.0	−127.8	−130.0	−127.6

Inosine and cytosine were attached to the monotonic backbone sequence of the right- and left-handed models of calf thymus B-DNA (3, 4, 8).

* As described by Arnott and Hukins (2).

† Total energy of interaction E was treated as $E = E_{1d} + E_{rep} + E_{es}$, where E_{1d} = energy due to London dispersion interaction between two atoms with finite sizes (unpublished result), E_{rep} = contribution from repulsion between two atoms with finite sizes (unpublished result), and E_{es} = energy due to monopole–monopole interactions. The base-pair geometries of the four models were kept fixed; thus, the H-bond energies that would be constant were not calculated separately. In the base-paired dinucleoside monophosphate, energy is expressed in units of kcal/2 mol (1 Cal = 4.18 J) of a dinucleoside monophosphate and total energy corresponds to kcal/2 mol of a trinucleoside diphosphate.

g^-g^-) conformation and, therefore, a low value of α (155°) and associated stereochemical problems. However, it is gratifying to note that recently Arnott *et al.* (9) abandoned the C2′-*endo*, g^-g^- model and chose a C2′-*endo*, tg^- model for B-DNA that is similar to the one we obtained by using a stereochemical guideline (5, 6).

Relative stabilities of the B- and D-DNA models of poly[d(I-C)]

As shown in Table 1, the right- and left-handed models for a given form of poly[d(I-C)] (i.e., B or D) have similar backbone torsion angles. However, the sugar–base orientations as determined by the glycosyl torsion χ and the stacking arrangements as decided by the handedness are different. The projection of the base-paired dinucleoside monophosphate fragments down the helix axis for the I-C and C-I sequences is shown in Fig. 2. The neighboring bases in the same strand tend to show greater geometric overlap in the B form of poly[d(I-C)] than in the D form. When energy values were computed for the four models, it was found that they were almost equally stable (Table 1 and Fig. 2). The observation that both right-handed and left-handed helical models of poly[d(I-C)] in a given form (B or D) are equally

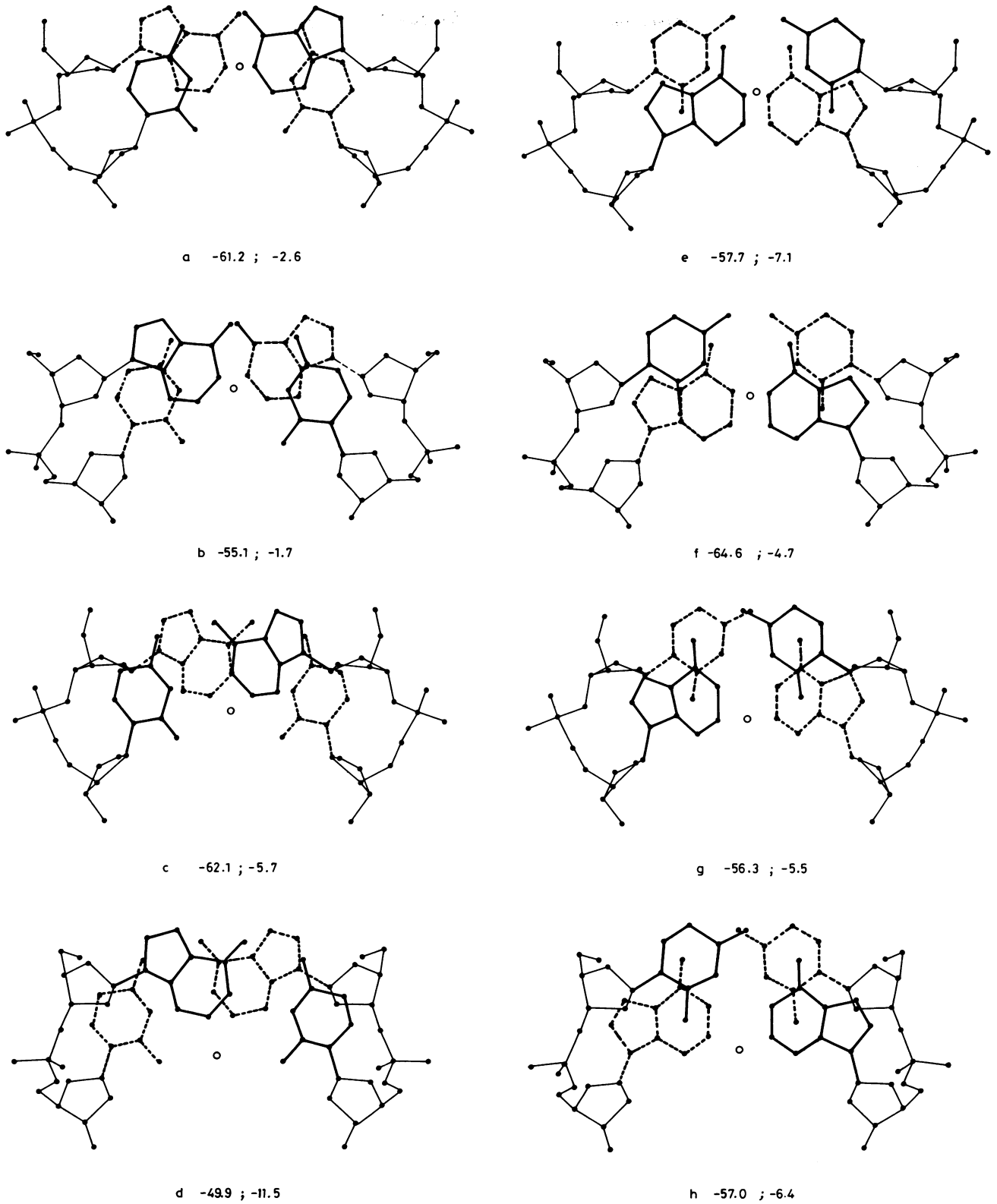

FIG. 2. Projections down the helix axis of the base-paired dinucleoside monophosphate fragments for I-C (*a–d*) and C-I (*e–h*) sequences. Intra- and interstrand interaction energies, including contributions from backbone, base–backbone, and base–base interactions (in kcal/2 mol of dinucleoside monophosphate) are shown below each projection. The bases on the top are marked by thick lines while those at the bottom are marked by dashed lines.

stable reaffirms our hypothesis that DNA can exist in either handedness and the fact that the B and D forms in a given handedness are equally stable is consistent with the smooth D → B transition in the solid phase.

Quantitative agreement of the right- and left-handed uniform helices in the D form

Both right-handed uniform and left-handed uniform helices of D-DNA have a molecular dyad between two neighboring base

Table 2. Structure-factor values for three DNA models

h	k	l	F_0^*	Uniform helix† Right	Uniform helix† Left	Arnott's model‡	h	k	l	F_0^*	Uniform helix† Right	Uniform helix† Left	Arnott's model‡
1	0	0	80.9	78.0	84.1	90.7	1	1	3	50.7	82.6	71.9	87.2
1	2	0	41.9	17.2	35.8	18.3	2	0	3	35.7	55.2	57.8	59.9
2	2	0	25.8	51.0	61.3	76.6	2	1	3	97.2	68.2	43.3	101.4
3	0	0	58.9	29.0	60.8	91.3	2	2	3	41.3	26.8	77.2	59.3
1	3	0	93.3	60.4	43.0	43.1	3	0	3	75.2	35.0	94.0	40.2
2	3	0	47.6	58.3	44.2	64.7	3	1	3	129.5	49.8	91.1	55.8
4	0	0	45.8	27.4	38.1	2.3	3	2	3	60.9	57.2	62.9	61.7
3	3	0	27.3	34.2	70.9	66.6	5	0	3	58.8	82.8	28.7	51.6
2	4	0	85.4	83.2	48.5	37.0	5	1	3	67.3	59.3	33.2	34.3
5	0	0	79.1	114.4	69.6	122.9	1	0	4	49.0	14.9	22.9	26.7
5	1	0	74.0	103.1	54.9	99.5	1	1	4	104.0	99.8	62.3	40.7
1	0	1	64.2	66.5	26.0	67.0	2	0	4	115.0	118.0	106.1	66.4
1	1	1	30.7	9.1	42.7	3.0	2	1	4	124.0	77.3	81.4	72.5
2	0	1	88.6	78.9	93.5	49.5	2	2	4	79.0	60.9	17.0	24.0
2	1	1	88.9	61.1	82.1	61.2	3	0	4	96.9	77.9	17.7	43.7
2	2	1	29.6	40.7	16.7	31.3	3	1	3	97.4	71.4	34.0	42.8
3	0	1	47.5	40.3	54.4	30.8	3	2	4	70.3	74.3	64.5	50.9
3	1	1	45.8	43.4	63.1	47.0	2	0	5	76.6	63.6	104.5	22.2
3	2	1	35.7	20.6	86.3	48.2	2	1	5	91.2	66.8	100.0	23.6
4	0	1	35.3	40.4	59.1	21.7	2	2	5	35.1	122.4	37.2	77.9
4	1	1	55.9	45.7	89.1	18.0	3	0	5	45.9	66.0	42.7	48.1
3	3	1	38.9	37.4	81.0	15.4	3	1	5	48.7	40.8	23.0	76.0
1	0	2	56.8	19.2	56.1	57.9	2	3	5	45.6	29.6	29.2	41.6
1	1	2	60.2	24.9	62.5	65.0	4	0	5	38.4	51.8	7.0	14.7
2	0	2	66.2	22.5	42.8	38.0	4	1	5	44.7	45.5	17.8	33.9
1	2	2	56.5	20.0	23.3	20.7	3	3	5	45.2	32.8	34.3	29.3
2	2	2	51.0	29.2	44.1	20.5	5	0	5	58.7	105.4	55.5	64.1
3	0	2	31.7	24.3	39.9	20.7	1	0	6	52.9	10.7	46.5	37.3
3	1	2	36.8	22.9	36.9	12.3	1	1	6	53.6	45.3	30.8	5.3
3	2	2	72.5	19.8	49.2	42.0	2	0	6	92.8	114.3	29.3	94.7
4	1	2	48.1	16.3	103.1	67.3	2	1	6	115.0	118.2	55.6	169.7
3	3	2	50.7	17.8	48.1	51.8	2	2	6	57.4	80.1	59.8	64.3
4	2	2	35.4	19.7	71.6	47.6	3	0	6	51.4	80.5	45.1	58.5
5	0	2	53.0	30.4	77.2	67.3	3	2	6	58.1	31.0	74.1	61.0
1	0	3	103.9	42.6	52.6	55.7							

* Arbitrary scale.
† (C2′-*endo*, tg^-).
‡ (C2′-*endo*, g^-g^-).

pairs. The dyad can be oriented by an angle θ between 0° and 22.5° with reference to the x-axis. The angle θ has a bearing on the packing arrangement and the calculated structure-factor amplitudes. The packing parameter θ was varied along with the other conformational parameters (see Table 1) so as to simultaneously satisfy the following three criteria: (*i*) allowed stereochemistry, (*ii*) favorable intermolecular packing, and (*iii*) low value of the crystallographic residual R.

The x-ray photograph showed a strong maximum near the meridian on the seventh layer line and a meridional reflection on the eighth layer line (Fig. 1*b*). However, the recording of intensity on higher layer lines ($l \geq 7$) proved rather difficult due to the very large spread of streaks across the layer line. Therefore, only those models for which the molecular transforms showed a strong maximum near the meridian on the seventh layer line and a meridional reflection (moderately strong) on the eighth layer line were subjected to the three criteria mentioned above. The right- and left-handed D-DNA models described in Table 1 gave R factors of 0.36 and 0.38, respectively. The values of the R factors are comparable with the R factors of different refined models of DNA and RNA as reported in the literature (10). The meager data did not warrant any refinement. The observed and calculated structure factors are given in Table 2.

Although the right-handed D-DNA model of Arnott *et al.* (2) was stereochemically unacceptable, it gave an R factor of 0.33 when the structure-factor amplitudes were computed and compared with the observed data for poly[d(A-T)]. However, it may be recalled that the D-form pattern of poly[d(A-T)] contained only 38 measurable reflections as against 69 for poly[d(I-C)] (compare Fig. 1 of the present paper and figure 1 of ref. 2). We therefore thought that it would be an interesting exercise to obtain a right-handed D-DNA model of poly[d(I-C)] in the D form with the (C2′-*endo*, g^-g^-) conformation suggested by Arnott *et al.* (2) and compute the R factor. In other words, we wanted to see whether a stereochemically unfavorable structure that gave good agreement with 38 reflections would lead to the same agreement with 69 reflections. We found that the D-DNA model with the (C2′-*endo*, g^-g^-) conformation that showed the best agreement with the 69 observed data gave an R factor of 0.42. Therefore, it can be concluded that the right-handed D-DNA model of Arnott *et al.* (2) is not only stereochemically unsatisfactory but also gives poor agreement with the observed fiber diffraction data for poly[d(I-C)].

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