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Structure of D-DNA: 8-fold or 7-fold helix?

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We have shown that both right- and left-handed uniform helical models (RU and LU models) could be built to give satisfactory agreement with the fibre diffraction data of poly[d(I-C)] in the D-form. Atomic coordinates of these two models are reported in the present work. Molecular transforms of these two models, as well as of the recently published Hoogsteen base-paired 7-fold helical structure of Drew and Dickerson, are given. In view of the work of Drew and Dickerson, attention is drawn to the presence of clear 004 and 008 reflections in the diffraction patterns of poly[d(I-C)] and poly[d(A-T)]. The available data strongly suggest an 8-fold helical structure for the D-form of DNA.
Key words: DNA/D-form/helical structure

Introduction

Mitsui *et al.* (1970) proposed a left-handed 8-fold helical structure with Watson-Crick base pairing for poly[d(I-C)] based on its X-ray fibre diagram and its unusual inverted c.d. pattern. This model was discarded by Arnott *et al.* (1974) on stereochemical grounds, especially its unusual O1′ endo sugar pucker. Instead, they proposed a right-handed model for poly[d(A-T)] in the D-form and also extrapolated this structure to poly[d(I-C)]. However, this structure was also not free of stereochemical problems, as pointed out earlier (Gupta *et al.*, 1980; Ramaswamy *et al.*, 1982). Later, it was shown in our laboratory that stereochemically satisfactory models of either handedness could be built to agree with the observed X-ray patterns of poly[d(A-T)] (Gupta *et al.*, 1980) and poly[d(I-C)] (Ramaswamy *et al.*, 1982).

Drew and Dickerson (1982) have proposed a left-handed Hoogsteen base-paired 7-fold helical structure for D-DNA. They argue that the strong intensity on the 7th layer line could be a true meridional and that the data published before their work was completed cannot resolve this question conclusively. They have further stated that the tetragonal symmetry of the lattice led investigators to explore only 8-fold helical models. The purpose of this paper is to show that the present available data on poly[d(I-C)] (Ramaswamy *et al.*, 1982) and poly[d(A-T)] (Mahendrasingam *et al.*, 1983) in fact clearly indicate an 8-fold structure for D-DNA rather than a 7-fold structure as proposed by Drew and Dickerson (1982).

Results and Discussion

Atomic coordinates of the right-handed uniform (RU) and left-handed uniform (LU) models for poly[d(I-C)] in the D-form proposed earlier (Ramaswamy *et al.*, 1982) are given in Table I. Both these models have the favorable correlation (Gupta *et al.*, 1982), namely C2′ endo sugar pucker and tg⁻ phosphate torsions. Our choice of 7-fold models was not based on the tetragonal symmetry of the lattice as claimed by Drew and Dickerson (1982). We have considered 8-fold models for the following two important reasons. (i) A precession photograph of poly[d(I-C)] clearly shows that the strong intensity on the 7th layer line is off meridional and that the true meridional is on the 8th layer line (Ramaswamy *et al.*, 1982). Densitometer traces of the poly[d(I-C)] data shown in Figure 1 clearly demonstrate that the strong 7th layer line intensity is off meridional and corresponds to the 107 reflection and that the true meridional is on the 8th layer line. (ii) There is a weak but unmistakable meridional reflection on the 4th layer line. The presence of this 004 reflection could be attributed to the alternating purine-pyrimidine sequence which makes the true repeat a dinucleotide. A 7-fold model such as that proposed by Drew and Dickerson (1982) clearly cannot account for the presence of this reflection. The above facts prompted us to consider only 8-fold models for D-DNA.

Further support for an 8-fold structure comes from the recent work of Mahendrasingam *et al.* (1983) who have published a series of diffraction patterns of poly[d(A-T)] under varying conditions of salt and humidity. Their D-pattern shows a striking resemblance to our own flat plate photograph of poly[d(I-C)] (Ramaswamy *et al.*, 1982). Although they have not taken a precession photograph, their flat plate picture itself shows that the true meridian is on the 8th layer line and that the strong intensity on the 7th layer line is off meridional. In fact, they have pointed out that the 7th layer line intensity is due to 107 and 117 reflections. Further, although they have not mentioned it, their diffraction pattern shows a very clear 004 reflection.

Molecular transforms of our RU and LU models for poly[d(I-C)] as well as of the 7-fold model of Drew and Dickerson (1982) are given in Figure 2. A detailed comparison of the transforms of the RU and LU models as well as of the 7-fold model of Drew and Dickerson (1982) with the X-ray data of poly[d(I-C)] shows that the agreement of our models and the 7-fold model is rather similar. The transform of the 7-fold model is rather similar to our LU model except of course on the 7th and 8th layer lines. This is not at all surprising since the 7-fold model has more or less the same gross features as the LU model. For example, the LU model has a phosphate radius of 7.58 Å while the two phosphate radii of the 7-fold model are 7.34 and 7.45 Å. The RU and LU models have chain separations of 6.85 and 6.66 Å, respectively, while the chain separation of the 7-fold model is ~6.85 Å. The base tilt of our LU model is near zero, similar to the 7-fold model. Our RU model has a base tilt of around −25° [following the sign convention of Arnott *et al.* (1969)]. Interestingly the transforms of both the RU and LU models show a strong off meridional on the 7th layer line as seen in Figure 2. Thus it would appear that this is an approximately cyclic phenomenon with the features of zero tilt (*viz.*, a strong 7th layer line) being reproduced at relatively high tilts of

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Table 1. Cylindrical polar coordinates of the RU and LU models for poly[d(I-C)] in the D-form

RU model				LU model			
Atom	*R*	ϕ	*Z*	Atom	*R*	ϕ	*Z*
03′	7.10	117.00	5.46	03′	7.07	118.81	−3.63
P	8.24	113.20	4.46	P	7.58	107.68	−4.17
O1P	8.63	119.70	3.44	O1P	9.03	108.22	−4.32
O2P	9.46	110.60	5.14	O2P	7.00	104.03	−5.42
O5′	7.64	103.65	3.78	O5′	7.30	99.50	−3.01
C5′	7.33	94.90	4.59	C5′	8.20	98.12	−1.91
C4′	6.48	86.30	3.90	C4′	7.55	92.00	−0.84
C3′	7.09	83.35	2.55	C3′	6.81	82.49	−1.42
C2′	6.20	90.30	1.60	C2′	5.36	86.98	−1.43
C1′	4.84	91.10	2.26	C1′	5.37	96.30	−0.19
O1′	5.14	91.60	3.66	O1′	6.78	99.21	−0.03
N9I	4.23	107.40	1.88	N9I	4.79	111.61	−0.16
C8I	5.07	120.70	2.03	C8I	5.74	122.45	−0.19
N7I	4.87	134.30	1.50	N7I	5.49	135.57	−0.15
C5I	3.60	135.30	0.99	C5I	4.15	138.68	−0.09
C6I	3.14	155.70	0.33	C6I	3.71	158.69	−0.02
N1I	1.81	158.30	−0.08	N1I	2.37	166.01	0.03
C2I	1.26	110.00	0.07	C2I	1.47	134.35	0.02
N3I	2.29	93.70	0.71	N3I	2.43	107.16	−0.04
C4I	3.10	113.90	1.25	C4I	3.56	120.07	−0.09
O6I	4.05	168.50	0.08	O6I	4.65	169.65	−0.01
03′	7.10	72.00	2.33	03′	7.07	73.81	−0.50
P′	8.24	68.20	1.33	P′	7.58	62.68	−1.04
O1P	8.63	74.70	0.33	O1P	9.03	63.22	−1.19
O2P	9.46	65.60	2.01	O2P	7.00	59.03	−2.29
O5′	7.64	58.65	0.65	O5′	7.30	54.50	0.12
C5′	7.33	49.90	1.46	C5′	8.20	53.12	1.22
C4′	6.48	31.30	0.77	C4′	7.55	47.00	2.29
C3′	7.09	38.35	−0.58	C3′	6.81	37.49	1.71
C2′	6.20	45.30	−1.53	C2′	5.36	41.98	1.70
C1′	4.84	46.10	−0.87	C1′	5.37	51.30	2.94
O1′	5.14	46.60	0.53	O1′	6.78	54.21	3.10
N1C	4.23	62.40	−1.25	N1C	4.79	66.61	2.97
C6C	5.15	74.50	−1.04	C6C	5.80	76.58	2.94
C5C	5.18	89.10	−1.40	C5C	5.80	90.04	2.97
C4C	4.12	98.60	−1.99	C4C	4.67	99.76	3.04
N3C	2.89	89.90	−2.18	N3C	3.40	93.34	3.07
C2C	3.01	64.62	−1.81	C2C	3.47	70.79	3.04
N4C	4.64	113.99	−2.36	N4C	5.16	114.22	3.07
O2C	2.49	43.20	−1.97	O2C	2.76	51.94	3.07

The complementary strand may be generated as follows:
For the RU model: add 22.5° to all the ϕ values and 1.565 Å to all the *Z* values and then change the signs of ϕ and *Z*.
For the LU model: add 22.5° to all the ϕ values and −1.565 Å to all the *Z* values and then change the signs of ϕ and *Z*.
Successive residues may be generated by applying a twist of 45° and a rise of 3.13 Å for the RU model and −3.13 Å for the LU model.

around −25°.

Structure factor calculations on the RU and LU models yielded R-factors of 0.36 and 0.38, respectively, as mentioned earlier (Ramaswamy *et al.*, 1982). Similar calculations on the 7-fold model of Drew and Dickerson (1982) using our diffraction data of poly[d(I-C)] gave an R-factor of ~0.38. Thus, the quantitative agreement of our models with the diffraction data of poly[d(I-C)] is also similar to that of the 7-fold helical model of Drew and Dickerson (1982).

Some points about the stereochemistry of the 7-fold model of Drew and Dickerson (1982) are worth mentioning. Drew and Dickerson have chosen a dinucleotide as the repeat in

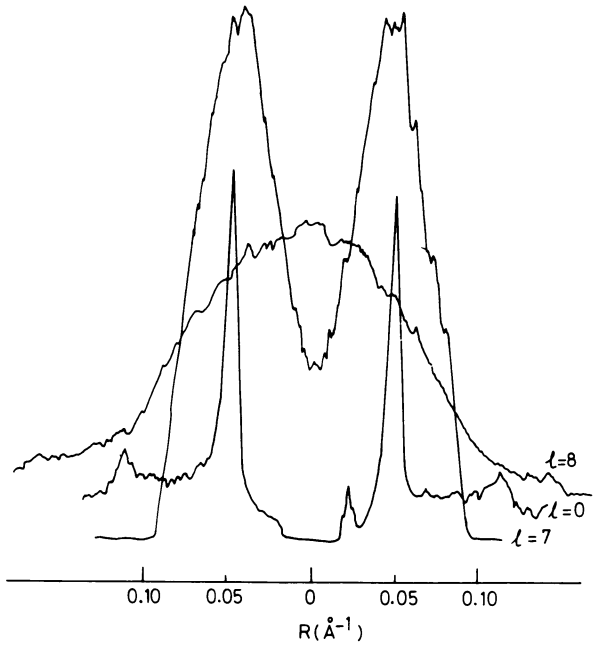

Fig. 1. Densitometer traces along layer lines 0, 7 and 8 of the flat plate photograph of poly[d(I-C)] in the D-form. Comparison of the equatorial and 7th layer line traces shows clearly that the strong intensity on the 7th layer line corresponds to the 107 reflection. The 8th layer line trace shows a clear meridional. Note that the three traces do not have a common base line. In the case of the 7th layer line, since it was extremely strong, a 5D filter was used.

their model. In general, a dinucleotide repeat should lead to better agreement indices because of the greater number of degrees of freedom available in a dinucleotide. The fact that there is a 004 reflection also suggests that the repeat is a dinucleotide. We have used a dinucleotide as the overall repeat but with a mononucleotide backbone repeat. At the present level of resolution we felt that a mononucleotide backbone repeat is a sufficient approximation and that the available data do not warrant any further refinement. However, in spite of using a mononucleotide backbone repeat, the quantitative fit of our models is similar to that of the dinucleotide model of Drew and Dickerson (1982) as pointed out above.

Conclusions

The X-ray fibre diffraction data of poly[d(I-C)] and poly[d(A-T)] point strongly to an 8-fold helical structure for the D-form of DNA. The presence of clear 008 and 004 meridional reflections and a strong off meridional 107 reflection lend support to this view. The strong off meridional on the 7th layer line can be reproduced by 8-fold models of either handedness. This is in contrast to the statement of Drew and Dickerson (1982) that 8-fold models could not account for a strong 7th layer. Any apparent agreement of the transform of the 7-fold model with the X-ray data is entirely due to the fact that the gross features of the model are nearly correct, and the data extend only up to ~4 Å. Thus, with the availability of good diffraction photographs of poly[d(I-C)]

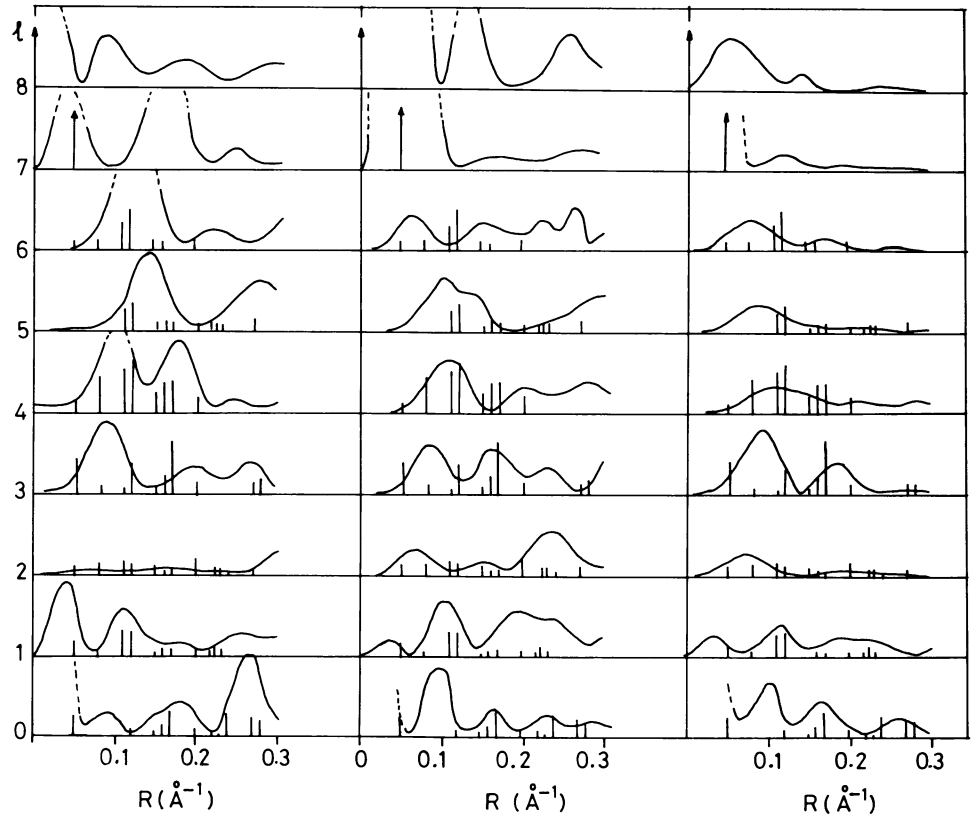

Fig. 2. Molecular transforms of our RU model **(left)**, our LU model **(middle)** and the 7-fold model of Drew and Dickerson (1982) **(right)** calculated using the water correction of Arnott and Hukins (1973). The observed X-ray intensity has been indicated by vertical bars on each layer line. Data beyond the 6th layer line are not included because the intensities were extremely strong and smeared. The reflections marked with an arrow were very strong and were not measured. The calculations on the RU and LU models were done using a c-axis repeat of 25.07 Å which corresponds to the pitch of the helix. The calculation on the 7-fold model was done assuming a c-axis repeat of 48.6 Å, which corresponds to twice the helix-pitch. Only even numbered layer lines, i.e., 0, 2, 4, 6, etc., are plotted in the case of the 7-fold model and these correspond respectively to layer lines 0, 1, 2, 3, etc., of the transforms of our 8-fold models.

(Ramaswamy *et al.*, 1982) and poly[d(A-T)] (Mahendrasingam *et al.*, 1983), we may altogether rule out a 7-fold structure for the D-form of DNA.

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