Acta Cryst. (1988). B44, 625–628

Low-Temperature Study of the A-DNA Fragment d(GGGCGCCC)

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(*Received* 16 *November* 1987; *accepted* 18 *April* 1988)

Abstract

The structure of the A-type duplex d(GGGCGCCC) was determined from data measured at 115 K to 2·0 Å resolution. The space group, $P4_32_12$, is the same as for the 293 K structure; cell dimensions are $a = 42{\cdot}74$ (4), $c = 24{\cdot}57$ (1) Å; $R = 0{\cdot}21$ for 1694 observed reflections. The conformation and hydration are similar at the two temperatures. The average displacement parameters (B) for bases, sugars and phosphates all decrease by about 9 Å^2 relative to those found at 293 K. The individual values of $B^{1/2}$ are linearly related to the distance from the molecular center of mass.

Introduction

It has been commonly assumed that crystals of biological macromolecules, with high water contents, cooled to low temperatures would undergo phase separation as a result of ice formation. Drew, Samson & Dickerson (1982) have described the results of slow cooling of a crystal of a B-DNA dodecamer. Although not explicitly stressed by these authors, the lack of phase separation is a strong indication that crystals of similar nature can in fact be cooled without loss of crystallinity.

Following the successful cryogenic treatment of crystals of crambin and BPTI by Hope (1988), a more extensive study of the effects of cooling on a variety of crystals of biological macromolecules was started in this laboratory. One of the compounds used in this study was the A-DNA octamer d(GGGCGCCC), the crystal structure of which had already been determined from room-temperature data (Rabinovich, Haran, Eisenstein & Shakked, 1988).

Experimental

A crystal of d(GGGCGCCC) of dimensions 0·4 × 0·1 × 0·1 mm was transferred from its mother liquor (30% v/v MPD, 50 mM cocodylate buffer, 15 mM $MgCl_2$) to a viscous hydrocarbon oil and shock-cooled to about 115 K, as described by Hope (1988). There was no indication of phase separation. However, the reflection profiles (ω scan) at low temperature were about twice as wide as the room-temperature ones. We have not yet determined the cause for this difference, which may have arisen either as a result of precooling manipulations, the cooling process itself, or the quality of the specimen.

The space group is tetragonal $P4_32_12$. Cell dimensions at room temperature (RT) [low temperature (LT)] are: $a = 43{\cdot}28$ [42·74], $c = 24{\cdot}66$ [24·57] Å, $V =$ 46 192 [44 882] Å^3, λ(Cu $K\alpha$) = 1·5418 Å. The measured decrease in volume of about 3% is typical for many molecular crystals.

To estimate the potential quality of data from this crystal, reflections were measured at a high speed (ω scan at 16° min^{-1}) on a Rigaku AFC5 diffractometer. The X-ray source was a Rigaku R300 rotating Cu anode operated at 15 kW and the Cu $K\alpha$ radiation was selected with a graphite monochromator. Three monitor reflections showed no variation beyond that expected from counting statistics and no change in crystal orientation was observed during the data collection.

Refinement of the structure was carried out in a similar manner to the room-temperature structure, using the restrained least-squares procedure of Hendrickson & Konnert (1981). The final R factor for the low-temperature data was 0·21, higher than that for the room-temperature refinement, 0·16.

Results

The intensity data

Of the 2299 reflections measured to $d = 1{\cdot}7$ Å, 1694 were observed [$F_o > 2\sigma(F_o)$]. The corresponding numbers in the room-temperature data set were 2455 and 2082. The resolution of the LT data was practically 2·0 Å because for higher resolution shells ($d < 2{\cdot}0$ Å) the majority of the reflections were unobserved (Table 1). Thus, in the range $2{\cdot}0 > d > 1{\cdot}7$ Å only 32% of the reflections were observed.

There appear to be two major but contradicting effects that determine the quality of the data set. The width of the peak profiles contributes toward a reduction in the peak-to-background ratio, whereas the lower temperature, with concomitant decrease in B values, contributes toward an increase in the peak-to-background ratio. Which of the two will consequently have the greater influence on the data depends on

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0108-7681/88/060625-04$03.00

Table 1. *Analysis of the number of observed* $[F > 2\sigma(F)]$ *reflections in small intervals in d*

	RT*			LT		
d_{min}	N(theory)	N(obs.)	%obs.	N(theory)	N(obs.)	%obs.
5·0	135	130	96	131	123	94
3·5	232	227	98	227	217	96
2·8	318	299	94	307	276	90
2·3	510	456	89	502	390	78
2·2	154	125	81	157	95	61
2·1	197	157	80	182	109	60
2·0	225	169	75	227	111	49
1·9	282	199	71	265	114	43
1·8	351	221	63	343	111	32
1·75	195	99	51	195	67	34
1·7				215	34	16

* RT data collection stopped at $d_{min} = 1{\cdot}75$ Å.

relative magnitudes in individual cases and on how well one compensates for peak broadening by increased measurement time. The poorer resolution of the LT data compared to the RT data (1·8 Å, Table 1) in the present case was initially related to the fast LT data collection. However, in a recent LT experiment, in which a crystal was shock-cooled and reflections were carefully scanned (Frolow, 1988), the resolution was essentially 2·1 Å, with only 28% observed reflections for $2{\cdot}1 > d > 1{\cdot}7$ Å. It appears that the cause for the limited LT resolution is the deterioration of the crystal upon cooling.

A plot of average $[F_o(\text{LT})]$/average $[F_o(\text{RT})]$ as a function of $\sin^2\theta/\lambda^2$ is shown in Fig. 1. All the theoretically possible reflections were included in calculating the intensity averages. The LT/RT ratio has a maximum of 1·25 at $\sin^2\theta/\lambda^2 \sim 0{\cdot}04$ ($d = 2{\cdot}5$ Å). The decrease in this ratio for higher $\sin^2\theta/\lambda^2$ reflects the larger proportion of unobserved reflections in the LT data set with respect to the RT one (Table 1).

Molecular geometry

The RT and LT structures are similar in terms of global and local conformational features (Table 2). The average helix twist and rise per base pair (which measure the relative rotation and displacement of successive base pairs with respect to the helix axis) are 31·5 [32·0]° and 3·3 [3·2] Å, respectively. Individual

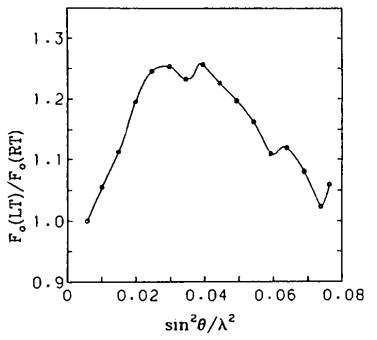

Fig. 1. The ratio average $[F_o(\text{LT})]$/average $[F_o(\text{RT})]$ in overlapping intervals in $\sin^2\theta/\lambda^2$.

helix-twist angles for the structures as well as individual values of rise per base pair are very close. The LT values of slide, which represents the displacement of successive base pairs along their long dimensions (Table 2), are slightly but systematically smaller than the RT values. This is a consequence of a slight shrinkage of the LT helix displayed as a shift of the base pairs toward the helix axis; the average perpendicular distances of the C6—C8 vectors from the best helix axes are 3·8 and 3·4 Å for the RT and LT structures, respectively. The values of roll and propeller twist, which measure the relative rotation of successive base pairs about their long axes and the relative rotation of the purine and pyrimidine bases within a base pair, differ in the two structures (Table 2). Discrepancies in these parameters, due to changes in temperature or crystal packing, have been noticed before by Dickerson, Kopka & Pjura (1985) for different variants of the B-DNA dodecamer and by Shakked & Rabinovich (1986) for A-type duplexes. The backbone torsion angles are similar in the two structures (Table 3).

A best molecular fit of the RT and LT structures results in an r.m.s. value of 0·36 Å. The largest deviations occur for the 3′-terminal deoxyribose.

Solvent structure

Positions of water molecules were deduced from $F_o - F_c$ electron density maps calculated for each data set. The number of water molecules per duplex located in the RT and LT structures is 88 and 62, respectively. Of these 64 [46] belong to the first hydration shell; the rest to the second shell. In the first hydration shell 38 waters are common to the two structures, suggesting a similar hydration arrangement at the two temperatures. Ten of the second-shell waters are also common.

Thermal parameters

As expected, the thermal parameters are appreciably smaller at low temperature, 17 Å^2 on the average *versus* 26 Å^2 at room temperature. The refined average B from the low-temperature data was slightly lower than the initial value calculated from a Wilson plot.

Individual B's were averaged for bases, sugars and phosphates giving 21·6 [12·5], 27·9 [19·5] and 32·4 [22·8] Å^2, respectively. If the variation of B with temperature is of the form $B(T) = B_0 + bT$, where B_0 is a 'zero-point' B and b is a proportionality constant (Hope, 1988), the quoted B's yield values of 6·6, 14·1 and 16·6 Å^2 for B_0 and 0·051, 0·047 and 0·054 Å^2 K^{-1} for b for the three categories, respectively. The B_0 values for sugars and phosphates are close to the average B's obtained by Drew, Samson & Dickerson (1982) at 16 K. Also in both experiments B(bases) < B(sugars) < B(phosphates). We interpret the large B_0 values as evidence of a static distribution of the molecules over a range of positions at low temperature,

Table 2. *Local conformational parameters at the two temperatures*

The first four parameters refer to base-pair doublets (numbered from 1 to 7) and the last refers to a single base pair.

Base pair	Helical step		Twist (°)	Rise (Å)	Slide (Å)	Roll (°)	Propeller twist (°)
G·C		RT					6·0
		LT					13·4
	1	RT	32·3	3·4	1·4	7·4	
		LT	32·5	3·3	1·3	9·9	
G·C		RT					10·5
		LT					8·8
	2	RT	31·2	3·2	1·7	6·0	
		LT	31·9	3·3	1·6	6·3	
G·C		RT					12·4
		LT					14·2
	3	RT	34·8	3·1	1·3	4·0	
		LT	35·4	3·0	1·1	4·1	
C·G		RT					7·8
		LT					8·5
	4	RT	23·9	3·3	2·2	3·1	
		LT	24·1	3·3	2·0	7·3	
G·C		RT					7·8
		LT					8·5
	5	RT	34·8	3·1	1·3	4·0	
		LT	35·4	3·0	1·1	4·1	
C·G		RT					12·4
		LT					14·2
	6	RT	31·2	3·2	1·7	6·0	
		LT	31·9	3·3	1·6	6·3	
C·G		RT					10·5
		LT					8·8
	7	RT	32·3	3·4	1·4	7·4	
		LT	32·5	3·3	1·3	9·9	
C·G		RT					6·0
		LT					13·4
Average		RT	31·5	3·3	1·6	5·4	9·2
		LT	32·0	3·2	1·4	6·9	11·2

Table 3. *Backbone parameters*

	α	β	γ	δ	ε	ζ	χ	P*	$P_i - P_{i+1}$†
RT	—	—	76	70	−156	−51	−172	10	—
LT	—	—	59	73	−130	−77	−173	17	—
RT	−83	179	58	69	−173	−52	−157	11	5·8
LT	−71	164	62	74	−150	−66	−169	10	6·1
RT	−96	174	86	76	−157	−77	−174	31	6·2
LT	−75	179	63	82	−163	−69	−165	28	6·3
RT	−62	181	53	79	−168	−80	−158	20	5·7
LT	−61	182	47	82	−164	−82	−150	15	5·6
RT	150	198	182	91	−138	−91	−175	6	6·9
LT	154	202	181	84	−144	−80	−172	10	6·9
RT	−59	164	54	78	−150	−77	−166	22	5·8
LT	−54	167	38	83	−156	−80	−165	6	5·7
RT	−61	176	56	81	−165	−60	−156	21	6·0
LT	−53	162	54	79	−154	−66	−163	21	6·0
RT	−66	172	50	94	—	—	−144	46	—
LT	−54	186	33	82	—	—	−145	18	—

* Pseudorotation phase angle. See definition by Saenger (1984).
† Distance between successive P atoms of the same strand (Å).

since a corresponding large-amplitude motion would be energetically improbable. At room temperature large-amplitude motion adds to the static disorder. The close similarity of the coefficients b for bases, sugars and phosphates suggests that the difference between the RT and LT average B's is mainly a result of a uniform decrease in the translational amplitudes of the molecule (see below).

Fig. 2 shows that at both temperatures the atomic $B^{1/2}$ are linearly related to the distance from the molecular center of mass. This suggests that the major contribution to the motion is of a rigid-body type or, in the low-temperature case, that the molecular conformation is largely preserved over the range of positions. The positive slopes in Fig. 2 indicate rigid-body librational amplitudes, whereas the LT and RT intercepts of 2·7 and 4·0 Å represent translational contributions of 7·3 and 16·0 Å^2, respectively, to the B's. It appears that the difference between the LT and RT translational amplitudes accounts for the difference between the corresponding average B values, 9 Å^2.

The average B's for bases, sugars and phosphates show similar variation at the two temperatures as illustrated in Fig. 3.

B parameters of water molecules were compared only for those common to the two structures. Their averages, 51·8 [36·0] Å^2, present a somewhat larger difference (15·8 Å^2) than that observed for the DNA atoms. At both temperatures the average B's are larger for the ten second-shell water molecules, 60·4 [42·3] Å^2, than for the 38 first-shell ones, 49·6 [34·3] Å^2. In the latter subset the 18 atoms that are hydrogen bonded to DNA bases have considerably lower B's, 43·1 [29·6] Å^2, than the 20 atoms hydrogen bonded to the backbone, 55·4 [38·6] Å^2. Thus, the static or dynamic disorder of the water molecules increases from the bases through the backbone to the second hydration shell. The differences between the average RT and LT B's for these groups follow a similar pattern; they are small for

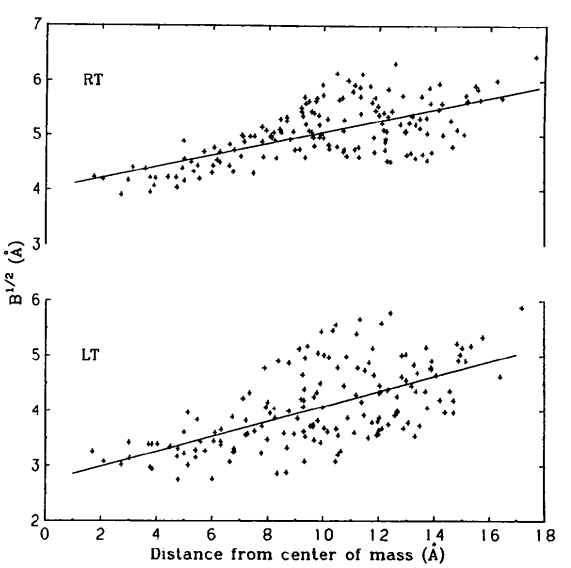

Fig. 2. Atomic $B^{1/2}$ *versus* distance from the molecular center of mass (R). The least-squares lines are given by 4·01 (10) + 0·105 (9) R (RT) and 2·72 (14) + 0·136 (14) R (LT).

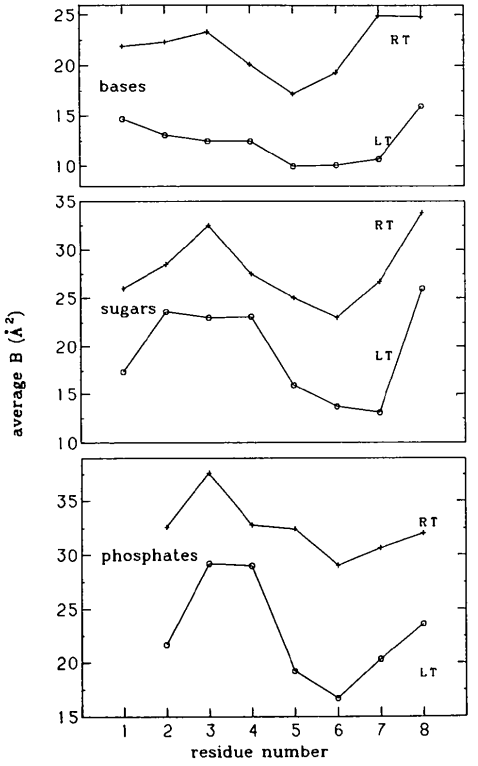

Fig. 3. The distribution of average B's for bases, sugars and phosphates.

the first group (13·5 Å^2) and larger for the second and third groups, 16·8 and 18·1 Å^2, respectively.

Concluding remarks

The successful cooling of several different DNA crystals by different techniques, as demonstrated here and elsewhere (Drew *et al.*, 1982), suggests that cryotechniques may be applicable to a wide variety of DNA crystals. The major advantage of data collection at cryotemperature is the apparent stability of the crystal. Since the preparation of suitable crystals is often a very difficult task, the need for fewer crystals may also be of considerable significance. The shock-cooling technique is experimentally very convenient compared to slow cooling over a period of days and, moreover, it eliminates all complications associated with the use of capillaries. However, this study and the study of d(GGGTACCC) (space group $P6_1$; Eisenstein, Frolow, Shakked & Rabinovich, in preparation) show that no improvement in resolution is achieved on applying cryotechniques to crystals of DNA fragments. Unlike crambin and BPTI, the damage caused to DNA crystals, which contain more than 50% solvent, outweighs the benefits of lowering the B values.

We thank the United States/Israel Binational Science Foundation (BSF) Jerusalem, Israel (grant No. 84-00264), the Fund for Basic Research administrated by the Israel Academy of Science and Humanities, and the Israel Cancer Association for financial support.

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Acta Cryst. (1988). B**44**, 628–636

Refinement of the Structure of Pseudoazurin from *Alcaligenes faecalis* S-6 at 1.55 Å Resolution

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(*Received* 26 *April* 1988; *accepted* 13 *July* 1988)

Abstract

The crystal structure of the redox protein pseudoazurin (123 amino acid residues; molecular weight 13 000 daltons) from *Alcaligenes faecalis* has been refined by fast Fourier restrained least-squares minimization. Cycles of rebuilding were carried out to escape from local minima. Individual isotropic temperature factor

0108-7681/88/060628-09$03.00