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Water mediated Dickerson-Drew-type crystal of DNA dodecamer containing 2′-deoxy-5-formyluridine

Masaru Tsunoda[a], Jiro Kondo[a], Naoko Karino[b], Yoshihito Ueno[b], Akira Matsuda[b], Akio Takenaka[a,*]

[a]*Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Midori-ku, Yokohama 226-8501, Japan*

[b]*Department of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University, Nishi 6, Kita 12, Kita-ku, Sapporo 060-0812, Japan*

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Abstract

To investigate the role of divalent cations in crystal packing, a Dickerson-Drew-type dodecamer with the sequence d(CGCGAATXCGCG), containing 2′-deoxy-5-formyluridine at X, was crystallized under several conditions with Ba^{2+} ion instead of Mg^{2+} ion. The crystal structure is isomorphous with the original Dickerson-type crystal containing Mg^{2+} ion. In the Mg^{2+}-free crystals, however, a five-membered ring of water molecules occupies the same position as the magnesium site found in the Mg^{2+}-containing crystals, and connects the two duplexes similarly to the hydrated Mg^{2+} ion. It has been concluded that the five-membered water molecules can take the place of the hydrated magnesium cation in crystallization. The 5-formyluracil residues form the canonical Watson–Crick pair with the opposite adenine residues.

Keywords: X-Ray structure; Damaged DNA; 5-Formyluridine; Water structure; DNA dodecamer

1. Introduction

At every step of biological systems, magnesium ions play important roles in the structure and function of nucleic acids, as the essential factors. The role of cations in crystallization of Dickerson-Drew-type oligonucleotides has been discussed with a new concept of phosphate group charge neutrality, which states that the oligonucleotide molecules are cemented at specific positions within the infinite crystal lattice by cations [1]. Depending on the size of the divalent cations, the crystal packing varies. Calcium ions facilitate the R3 form by binding in the minor groove [2,3], while magnesium ions stabilize the well known $P2_12_12_1$ form by coordinating in the major groove of the two duplexes [1,4–10]. So far, divalent cations have been regarded as essential for crystallization of the Dickerson-Drew-type oligomers, particularly magnesium cations for the $P2_12_12_1$ form. There have been no reports of crystallization without divalent

*Corresponding author. Tel.: +81-45-924-5709; fax: +81-45-924-5748.

E-mail address: atakenak@bio.titech.ac.jp (A. Takenaka).

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cations. To examine the role of the divalent cations, we have determined the crystal structure of a DNA dodecamer with the sequence d(CGCGAATXCGCG), containing 2′-deoxy-5-formyluridine at X.

An additional purpose of the present work is to reveal the hydrogen bond scheme of the 5-formyluracil residue in *B*-form DNA duplex, because activated O atoms, such as those in hydroxyl radicals, hydrogen peroxide and hydrogen superoxide anion radicals, are known to be potent mutagens, which produce damage in the DNA [11]. Thymine bases are oxidized at their 5-methyl group to 5-formyluracil [12]. This change will induce ionization of O^4 so that the formyl group, which is an electron-withdrawing group, increases the acidity of the N^3-proton of the base moiety [13]. It has been demonstrated from in vitro polymerization that 2′-deoxy-5-formyluridine triphosphate is incorporated into a synthesized DNA strand when the template is a guanine residue [13] with the same efficiency as when the template is the complementary adenine residue.

2. Materials and methods

2′-Deoxy-5-(1,2-dihydroxyethyl)uridine amidite was synthesized from 5-iodo-2′-deoxyuridine according to the reported method [14] with a slight modification, and was then incorporated at the eighth position of the present dodecamer on a DNA synthesizer.

Divalent cations were removed using a Chelex 100 resin (Bio-Rad Laboratories). Crystallization conditions were surveyed in 20 mM sodium cacodylate buffer (pH 6–7) by changing the concentrations of DNA dodecamer, spermine tetrahydrochloride, barium and sodium cations and 2-methyl-2,4-pentanediol using hanging-drop and sitting-drop vapour diffusion methods. Within a week, single crystals were obtained at 277 K under the condition as given in Table 1. They have a rod shape, with dimensions of roughly $1.0\times0.3\times0.3$ mm.

X-Ray diffraction data of the crystals were collected at 100 K by the oscillation method on the Sakabe Weissenberg camera [15] with synchrotron radiation ($\lambda = 1.00$ Å) at the Photon Factory in Tsukuba. Diffraction patterns were processed with the program DENZO [16]. Initial phases were derived by the molecular-replacement method with the program AMoRe [17] using the atomic coordinates of the dodecamer $d(CGCGAATTCGCG)_2$ [6] as a probe. The molecular structure was constructed and modified on electron density maps with the program QUANTA (Molecular Simulations Inc., San Diego, USA). The atomic parameters were refined with the program CNS [18]. The crystallographic data, data collection and structure determination statistics are given in Table 2. The atomic coordinates have been deposited in the Protein Data Bank (PDB) with the ID code of 1g8u.

Table 1
Initial condition for crystallization

Droplets	
DNA	0.5 mM
Na cacodylate	20 mM, pH 7.0
Spermine 4HCl	6 mM
Divalent cation	10 mM Ba^{2+}
Monovalent cation	40 mM Na^{+}
MPD	5%
Reservoir	
MPD	40%

MPD: 2-methyl-2,4-pentanediol

3. Results and discussion

3.1. Overall structure

Fig. 1 shows an overview of two dodecamers (chains a and b) which are associated to form a right-handed double helix as expected. All local helical parameters, calculated by the program NUPARAM [19], are given in Table 3. To detect the influence of the formyl group on the DNA conformation, the two representative parameters, rise and displacement, are plotted along the nucleotide sequences (see Fig. 2) for the present dodecamer and for the refined original Dickerson-Drew-type DNA dodecamer [20]. These parameters fluctuate around average values close to those of the typical *B*-form DNA, and their patterns are very similar to each other, indicating

Table 2
Crystallographic data and structure-determination statistics

Crystal data	
Space group	$P2_12_12_1$
Cell constants (Å)	
–*a*	25.2
–*b*	41.2
–*c*	65.4
–*Z*[a]	1
Data collection	
Limiting resolution (Å)	1.7
Observed reflections	31872
Independent reflections	7779
R merge (%)	2.9
Completeness (%)	97.5
Structure refinement	
Non-H DNA atoms	488
Water molecules	82
Reflections used for refinement	5910
Resolution range (Å)	50–1.85
R factor (%)	24.0
R free (%)[b]	27.5
R.m.s deviations	
–Bond lengths (Å)	0.004
–Bond angles (°)	0.9
–Improper angles (°)	1.3
Average coordinate error (Å)	0.26

[a] Number of duplexes in the asymmetric unit.
[b] Calculated using the 10% of reflection data which were not used for refinement.

that the formyl group gives no significant changes in the overall DNA conformation.

3.2. Crystal packing

The present dodecamer duplex makes a direct contact with another duplex related by crystallographic 2_1 symmetry to form an extended column along the *c* axis in a head to tail fashion. Two guanine–guanine extra pairs, G^{i}_{a12}:G^{ii}_{a2} and G^{i}_{b2}:G^{ii}_{b12}, occur between one end of a duplex (i) and the other end of another duplex (ii) through two N^2-H···N^3 hydrogen bonds for each pair. Fig. 3 shows the details of these hydrogen bonds. This direct contact is a common feature for $P2_12_12_1$ crystals of Dickerson-Drew-type dodecamers [1,4–10,20,21].

Here it is interesting to compare the two crystal structures: one that is obtained from Mg^{2+} free-solutions (present work); and those obtained from Mg^{2+} contained solutions [1,4–10,20,21]. Fig. 4 shows a typical example of the octahedrally hydrated magnesium cation found in the major groove of the duplex. The six water molecules coordinated around the magnesium cation form hydrogen bonds to join the two duplexes related by 2_1 symmetry along the *b* axis. Three hydrogen bonds occur between OW^1 and the O^6 atom of G_{b10}, between OW^2 and the O^6 atom of G_{a2}, and between OW^3 and the N^7 atom of G_{a2} (see the

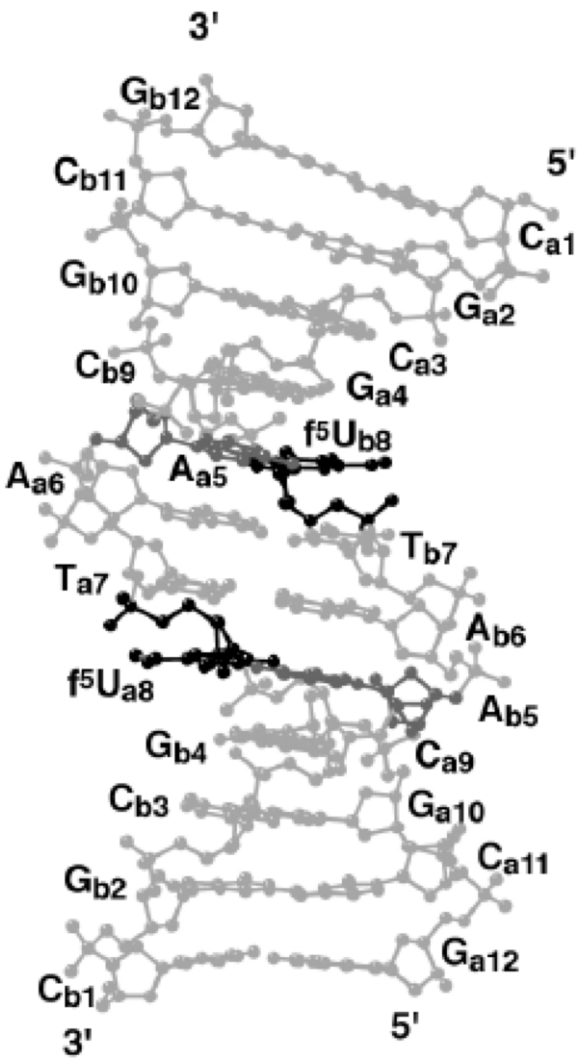

Fig. 1. An overview of the duplex structure of DNA dodecamer containing 2′-deoxy-5-formyluridine at the eight position. The two chains are designated as a-chain and b-chain.

Table 3
Local helical parameters

	Base pair	Tilt	Roll	Twist	Displacement	Rise
1	C_{a1}:G_{b12}	−2.56	−3.37	39.2	1.19	3.22
2	G_{a2}:C_{b11}	2.17	−0.04	36.4	0.97	3.39
3	C_{a3}:G_{b10}	2.00	10.6	30.0	0.59	3.58
4	G_{a4}:C_{b9}	0.70	1.74	33.4	0.69	3.17
5	A_{a5}:f^5U_{b8}	−0.11	1.15	37.3	0.71	3.16
6	A_{a6}:T_{b7}	−0.30	−0.24	34.0	0.14	3.20
7	T_{a7}:A_{b6}	0.02	−3.00	34.8	0.80	3.13
8	f^5U_{a8}:A_{b5}	1.89	−0.37	39.7	0.77	3.12
9	C_{a9}:G_{b4}	−2.02	4.18	30.2	0.56	3.48
10	G_{a10}:C_{b3}	−3.04	−10.4	42.0	1.76	3.25
11	C_{a11}:G_{b2}	0.92	3.89	35.5	0.96	3.38
12	G_{a12}:C_{b1}					
	Average	−0.03	0.38	35.7	0.83	3.28
	S.D.	1.75	5.00	3.6	0.39	0.15

Values of tilt, roll and twist are in degree and those of displacement and rise are in Å.

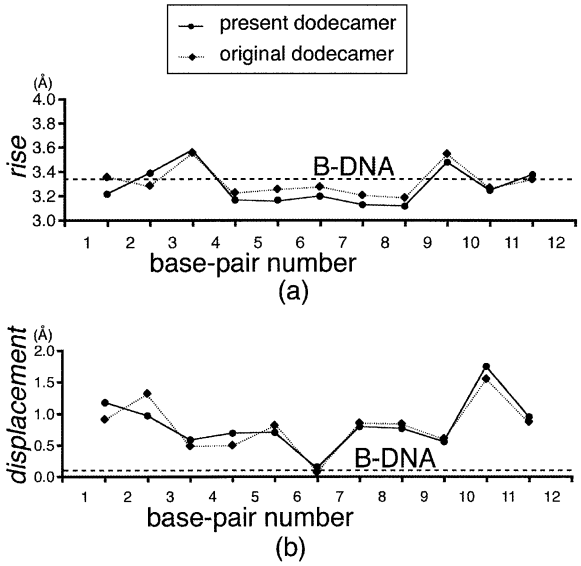

Fig. 2. Helical parameters, rise and displacement, plotted along the base-paired sequence. The atomic coordinates of the original dodecamer were obtained from PDB 1FQ2(NDB BD0041) [20].

nucleotide numbering in the Fig. 1). These residues are in one duplex. The other three hydrogen bonds from atoms OW^4, OW^5 and OW^6, are pointed to the phosphate oxygen atoms of the $T_{a7'}$ and $A_{a6'}$ residues in the adjacent duplex. This

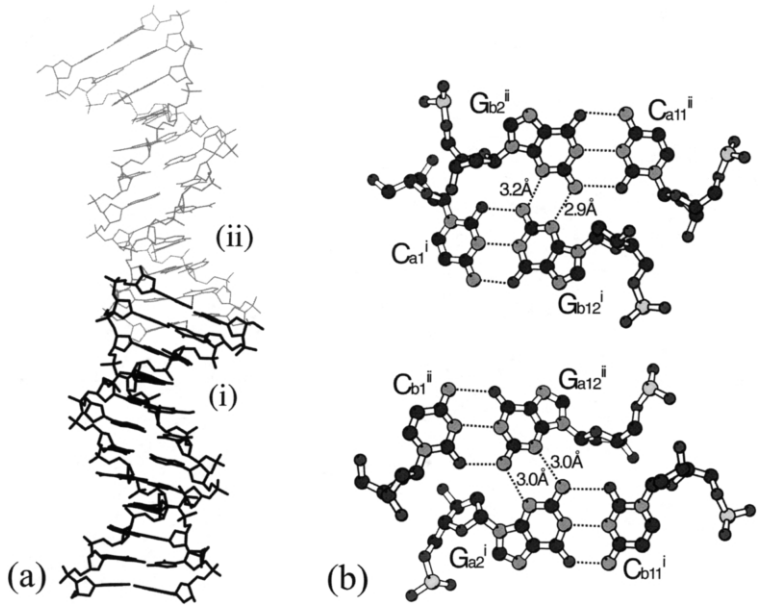

Fig. 3. Hydrogen bonded geometry of DNA–DNA direct contact through G:G pairing. The two duplexes, i and ii, related by crystallographic 2_1 symmetry along the *c* axis, make contact at the two G:G pairs (a). Each pair is formed through two N^2-H···N^3 hydrogen bonds (b).

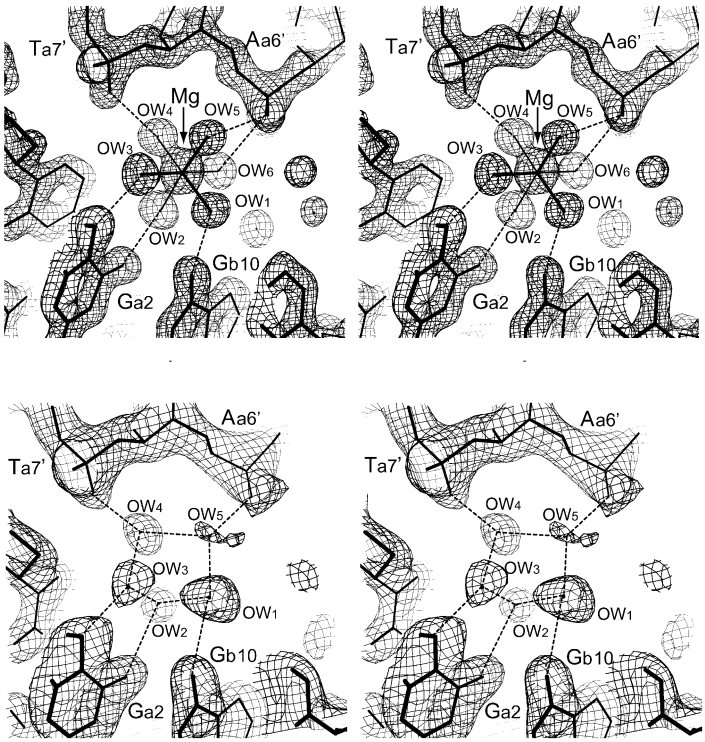

Fig. 4. Stereo-diagrams of $2|Fo|-|Fc|$ electron densities: (a) around the octahedrally hydrated magnesium cations found in the Mg^{2+}-containing crystal [21], contoured at the 2σ level; and (b) around the five-membered ring of water molecules connecting the two DNA duplexes, contoured at the 1σ level. The two linking groups are found at almost the same positions. In (b), the fifth water molecule, OW^5, has weak electron density owing to its disorder.

linkage of duplexes has been found in the Dickerson-Drew-type $P2_12_12_1$ crystals as a common feature [1,4–10,20,21]. Recently we have found a similar linkage in the crystal of the present DNA dodecamer containing 2′-deoxy-5-formyluridine, when it was crystallized in Mg^{2+} containing solutions [21].

The present crystal is isomorphous to the other Dickerson-type crystals, having the same space group and almost the same unit-cell parameters, in spite of that the former crystal are obtained from a solution containing Ba^{2+}, but no Mg^{2+}. In the present crystal, five water molecules, OW^1, OW^2, OW^3, OW^4 and OW^5, are located near the site of Mg^{2+} binding found in the other crystals (see Fig. 4). They apparently form a five-membered water ring through hydrogen bonds, although one of them is weakly ordered. Furthermore, it is interesting to note that these water molecules participate in connecting the two duplexes related by 2_1 symmetry along the *b* axis, in a similar way to the hydrated Mg^{2+}. As shown in Fig. 4, similar hydrogen bonds occur between OW^1 and the O^6 atom of G_{b10}, OW^2 and the O^6 atom of G_{a2}, OW^3 and the N^7 atom of G_{a2}, OW^4 and the phosphate oxygen atom of $T_{a7'}$, and OW^5 and the phosphate oxygen atom of $A_{a6'}$. As compared with the Mg^{2+}-containing crystals, the positions of the five water molecules are close to those of the six water molecules, and only OW^6 is missing. The present work indicates that the five-membered water molecules can take the place of the hydrated magnesium cation in DNA–DNA interaction, suggesting that not only the magnesium cation but also the water molecules have an important role in DNA folding, as a cement between duplexes.

Water molecules found in the minor and the major grooves and those around the phosphate

groups occupy similar positions to those of the Mg^{2+}-containing crystals with some differences, and some of them are similar to those of the original dodecamers. In this crystal, barium ions are not found, but may be involved in neutralizing the negative charges of the phosphate groups. Sodium cations and spermine may also contribute to such neutralization.

3.3. Hydrogen-bonding scheme of f^5U:A base-pairs

The second interest of the present work is focussed to the 5-formyluracil residue. It is important to examine if the modified base still has an ability to form the canonical Watson–Crick base pair with the opposite adenine residue in the *B*-form duplex, because DNA polymerases can accept only such base pairs. At the two positions of the duplex, f^5U_{a8} and f^5U_{b8}, the 5-formyluracil residues form a pair (see Fig. 5) with the opposite A_{b5} and A_{a5} residues in the Watson–Crick geometry, respectively. The distances from $O^4(f^5U_{a8})$ to $N^6(A_{b5})$, and from $N^3(f^5U_{a8})$ to $N^1(A_{b5})$ are 3.13 and 2.79 Å, respectively, and those from $O^4(f^5U_{b8})$ to $N^6(A_{a5})$, and from $N^3(f^5U_{b8})$ to $N^1(A_{a5})$ are 2.93 and 2.72 Å, respectively. These must be hydrogen bonds. To form them, both the f^5U_{a8} and f^5U_{b8} residues should be in imino form. For this pairing, the formyl group of f^5U_{b8} adopts a syn conformation to the C^4 atom around the C^5–C^{5M} bond, while the formyl group of f^5U_{a8} is disordered between the syn and anti conformations with almost equal occupancies. It is thus concluded that 5-formyluracil can form a canonical Watson–Crick base pair with adenine in DNA in the same way as thymine.

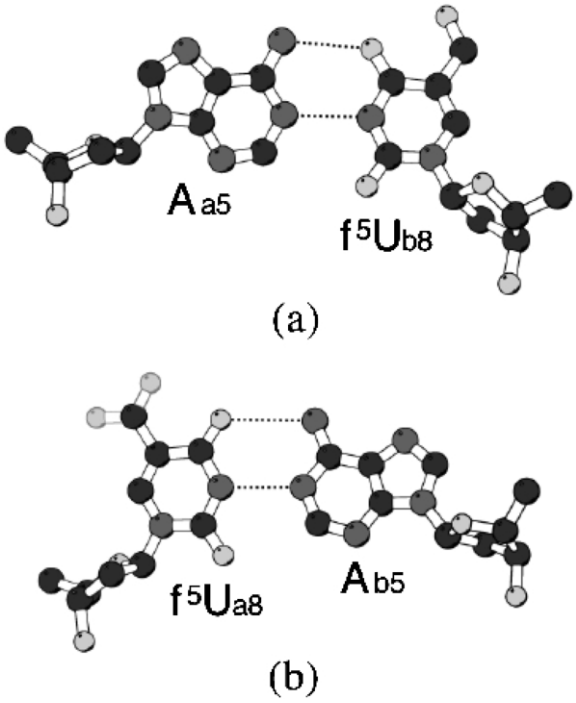

Fig. 5. Watson–Crick type hydrogen bonded pattern of f^5U:A pairs: (a) for f^5U_{b8}; and (b) for f^5U_{a8}. The formyl O atom of f^5U_{b8} adopts a syn conformation to the C^4 atom around the C^5–C^{5M} bond, while that of f^5U_{a8} is disordered between syn and anti conformations with almost equal occupancies.

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