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Crystallization and preliminary X-ray analysis of a DNA dodecamer containing 2′-deoxy-5-formyluridine; what is the role of magnesium cation in crystallization of Dickerson-type DNA dodecamers?

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To investigate the role of divalent cations in crystal packing, four different crystals of a Dickerson-type dodecamer with the sequence d(CGCGAAT*X*CGCG), containing 2′-deoxy-5-formyluridine at *X*, were obtained under several conditions with and without divalent cations. The crystal structures are all isomorphous. The octahedrally hydrated magnesium cations found in the major groove cement the two neighbouring duplexes along the *b* axis. In the Mg^{2+}-free crystals, a five-membered ring of water molecules occupies the same position as the magnesium site and connects the two duplexes similarly to the hydrated Mg^{2+} ion. It has been concluded that water molecules can take the place of the hydrated magnesium cation in crystallization, but the magnesium cation is more effective and gives X-ray diffraction at slightly higher resolution. In all four crystals, the 5-formyluracil residues form the canonical Watson–Crick pair with adenine residues.

1. Introduction

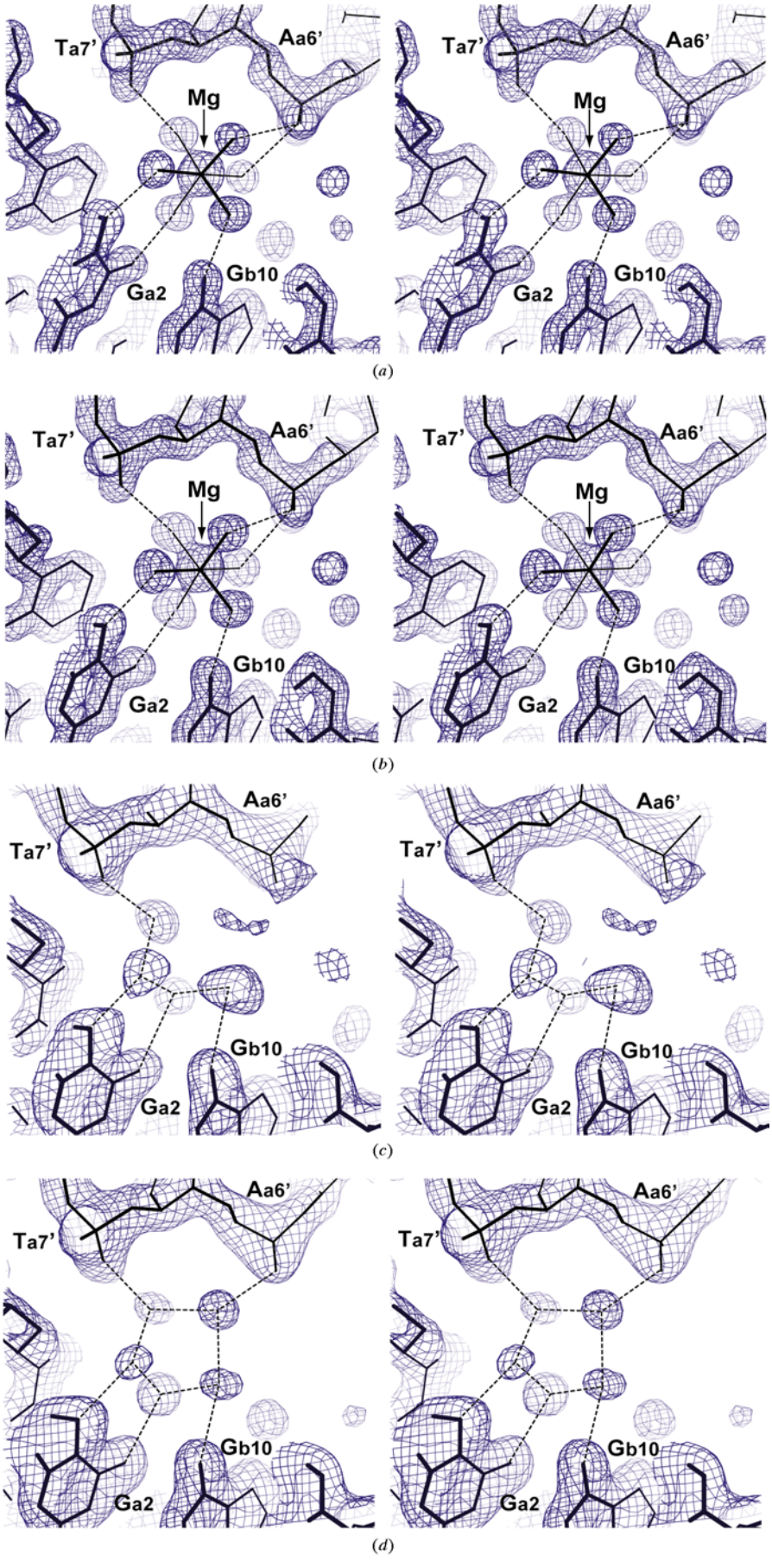
Magnesium ions play important roles in the structure and function of nucleic acids. The role of cations in crystallization of Dickerson-type oligonucleotides has been discussed with a new concept of *phosphate group charge neutrality*, which states that the oligonucleotide molecules are cemented at specific positions within the infinite crystal lattice by cations (Chiu *et al.*, 1999). Depending on the size of the divalent cations, the crystal packing varies. Calcium ions facilitate the *R*3 form by binding in the minor groove (Liu & Subirana, 1999; Minasov *et al.*, 1999), while magnesium ions stabilize the well known $P2_12_12_1$ form by coordinating in the major groove of the two duplexes (Pjura *et al.*, 1987; Quintana *et al.*, 1991; Shui *et al.*, 1998; Chiu *et al.*, 1999; Chatake, Hikima *et al.*, 1999; Chatake, Ono *et al.*, 1999; Hikima *et al.*, 1999; Hossain *et al.*, 2000). So far, divalent cations have been regarded as essential for crystallization of the Dickerson-type oligomers, particularly magnesium cations for the $P2_12_12_1$ form. There have been no reports of crystallization without divalent cations.

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 (Breen & Murphy, 1995). Thymine bases are oxidized at their 5-methyl group to 5-formyluracil (Kasai *et al.*, 1990). 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 (Yoshida *et al.*, 1997). 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 (Yoshida *et al.*, 1997) with the same efficiency as when the template is the complementary adenine residue.

In a series of crystallographic studies on damaged DNA (Chatake, Hikima *et al.*, 1999; Chatake, Ono *et al.*, 1999; Hikima *et al.*, 1999; Hossain *et al.*, 2000), a Dickerson-type dodecamer with the sequence d(CGCGAAT*X*CGCG) containing an oxidized thymine residue, 2′-deoxy-5-formyluridine (hereafter designated as ^{f5}U), at *X* has been synthesized and crystallized under several different conditions with and without divalent cations. Four different crystals have been obtained and their crystal structures given a preliminary examination. This paper considers the significance of divalent cations.

2. Materials and methods

2′-Deoxy-5-(1,2-dihydroxy)uridine amidite was synthesized from 5-iodo-2′-deoxyuridine according to the method reported by Sugiyama *et al.* (1996) 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 m*M* sodium cacodylate buffer (pH 6–7) by changing the concentrations of DNA dodecamer, spermine tetrahydrochloride, divalent cations, monovalent 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 four different conditions as given in Table 1. They have a rod shape, with dimensions of roughly 1.0 × 0.3 × 0.3 mm.

X-ray diffraction data from the four crystals, *A*, *B*, *C* and *D*, were collected at 100 K by the oscillation method on the Sakabe–Weissenberg camera (Sakabe, 1991) with synchrotron radiation (λ = 1.00 and 0.90 Å) at the Photon Factory in Tsukuba. Diffraction patterns were processed by the program *DENZO* (Otwinowski & Minor, 1997). Initial phases were derived by the molecular-replacement method with the program *AMoRe* (Navaza, 1994) using the atomic coordinates of the dodecamer d(CGCGAATTCGCG)$_2$ (Shui *et al.*, 1998) as a probe. The molecular structures were 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* (Brunger *et al.*, 1998). The crystallographic data and structure-determination statistics of the four crystals are given in Table 2.

3. Results and discussion

The four crystals *A*, *B*, *C* and *D* are mutually isomorphous, having the same space group and almost the same unit-cell parameters. The dodecamers are associated into B-form duplexes[1] in all crystalline forms and have no large conformational differences between them. In these crystals, the DNA duplexes make contact at both ends with

[1] The two chains are designated as a-chain and b-chain according to Dickerson's nomenclature (Drew *et al.*, 1981; Dickerson & Drew, 1981).

Figure 1
Stereodiagrams of $2|F_o| - |F_c|$ electron densities around the octahedrally hydrated magnesium cations connecting the two DNA duplexes (*a*) in crystal *A* and (*b*) in crystal *B*, contoured at the 2σ level, and those around the five-membered ring of water molecules connecting the two DNA duplexes (*c*) in crystal *C* and (*d*) in crystal *D*, contoured at the 1σ level. The two linking groups are found at almost the same positions. In crystal *C*, one water molecule has weak electron density owing to its disorder.

Table 1
Initial conditions for crystallization.

Crystal form	*A*	*B*	*C*	*D*
Droplets				
DNA (m*M*)	0.5	0.5	0.5	0.5
Sodium cacodylate	20 m*M*, pH 6.5	20 m*M*, pH 6.0	20 m*M*, pH 7.0	20 m*M*, pH 6.0
Spermine.4HCl (m*M*)	6	6	6	6
Divalent cation	6 m*M*, Mg^{2+}	10 m*M*, Mg^{2+}	10 m*M*, Ba^{2+}	—
Monovalent cation	6 m*M* Na^+ + 60 m*M* K^+	40 m*M* Na^+	40 m*M* Na^+	6 m*M* Na^+ + 40 m*M* K^+
MPD (%)	5	5	5	5
Reservoir				
MPD (%)	40	40	40	40

Table 2
Crystallographic data and structure-determination statistics.

Crystal form	*A*	*B*	*C*	*D*
Crystal data				
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters				
a (Å)	25.9	26.0	25.2	25.3
b (Å)	39.3	39.5	41.2	41.7
c (Å)	65.0	65.7	65.4	66.0
Z†	1	1	1	1
Data collection				
Limiting resolution (Å)	1.57	1.5	1.7	1.8
Observed reflections	87588	66227	31872	45728
Independent reflections	9502	10301	7779	6883
R_{merge} (%)	3.1	4.8	2.9	2.8
Completeness (%)	97.1	99.8	97.5	99.1
Structure refinement				
Non-H DNA atoms	488	488	488	488
Water molecules	150	185	82	145
Ions	1 Mg^{2+}, 1 K^+	1 Mg^{2+}	—	—
Reflections used for refinement	9313	9956	5910	6709
Resolution range (Å)	50–1.57	50–1.55	50–1.85	50–1.8
R factor (%)	21.0	20.7	24.0	20.8
R_{free}‡ (%)	23.6	23.1	27.5	23.9
R.m.s deviations				
Bond lengths (Å)	0.004	0.004	0.004	0.004
Bond angles (°)	0.9	0.9	0.9	0.9
Improper angles (°)	1.3	1.3	1.3	1.2
Average coordinate error (Å)	0.23	0.22	0.26	0.23

† Number of duplexes in the asymmetric unit. ‡ Calculated using the 10% of reflection data which were not used for refinement.

neighboring duplexes related by 21 symmetry along the *c* axis. Two extra base pairings occur through N(2)—H···N(3) hydrogen bonds between guanine bases of different duplexes. This packing motif is the same as that reported for the Dickerson dodecamer (Drew *et al.*, 1981; Dickerson & Drew, 1981).

In crystal *A*, a potassium ion in the minor groove on a pseudo-twofold axis of the palindromic dodecamer is bound to the O(2) atoms of two thymine residues, Ta7 and Tb7. In both crystals *A* and *B*, a magnesium cation is found in the major groove, as expected from the crystallization conditions. The magnesium cation is octahedrally coordinated by six water molecules and bound to the Ga2 residue and to the Gb10 residue of the same duplex through three hydrogen bonds from the hydrated waters to the N7 and O6 atoms and the O6 atoms of the two guanine residues, as seen in Figs. 1(*a*) and 1(*b*). At the same time, the other hydrated waters form three hydrogen bonds with the phosphate groups of the neighboring duplex, linking the two duplexes along the *b* axis. This connection suggests that the magnesium cation is essential for Dickerson-type $P2_12_12_1$ crystals.

In contrast, in crystal *C* obtained from a solution containing Ba^{2+} but no Mg^{2+}, several water molecules are found near the site of Mg^{2+} binding in crystals *A* and *B* (see Fig. 1*c*). They apparently form a five-membered water ring, although one of them is weakly ordered. The water ring joins the two duplexes in a similar way to the hydrated Mg^{2+}. In this crystal, barium ions are not found, but may be involved in neutralizing the negative charges of the phosphate groups. Monovalent cations and spermine may also contribute to such neutralization.

Crystal *D* was obtained from the completely divalent cation-free solution. In this crystal, five water molecules clearly form a five-membered ring and occupy positions similar to those found in crystal *C* (see Fig. 1*d*). Compared with crystals *A* and *B*, the water ring is located at the same position as the hydrated magnesium cation. It is interesting to note that the five water molecules form similar hydrogen bonds connecting the two duplexes. Crystal *D* suggests that the Dickerson-type DNA dodecamer might be crystallized in the $P2_12_12_1$ form without any divalent cations.

It is expected that the hydrated magnesium cation is a stronger connector between duplexes than the five-membered water ring. The temperature factors of phosphate groups were compared between the four crystals, assuming full occupancies. Crystals *A* and *B* have lower values than crystals *C* and *D*, the averaged temperature factors being 21.7, 20.4, 36.5 and 31.3 $Å^2$, respectively. Furthermore, in crystals *A* and *B* individual temperature factors are much lower at residues Aa6 and Ta7, whose phosphate groups interact directly with the hydrated magnesium cation. It is thus concluded that the five-membered water ring can take the place of the hydrated magnesium cation in crystallization of the $P2_12_12_1$ form, but the magnesium cation is more effective and gives X-ray diffraction at slightly higher resolution.

Although the four crystals were obtained under different conditions, the two formyl groups of residues ^{f5}Ua8 and ^{f5}Ub8 are in similar situations in these crystals, despite the different molecular environments mentioned above. The formyl group of ^{f5}Ub8 adopts a *syn* conformation to the C(4) atom around the C(5)—C(5M) bond, while the formyl group of ^{f5}Ua8 is disordered between the *syn* and *anti* conformations with almost equal occupancies. In all of these crystals, the two ^{f5}U residues form a

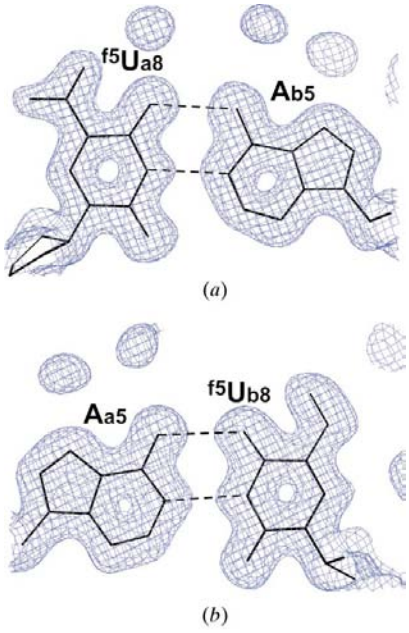


Figure 2
$2|F_o| - |F_c|$ electron densities of 5-formyluracil residues paired with adenine residues, contoured at the 1σ level. ^{f5}Ua8 (*a*) and ^{f5}Ub8 (*b*) residues are paired with Ab5 and Aa5 residues, respectively, in the Watson–Crick manner. The formyl O atom of residue ^{f5}Ua8 is disordered between *syn* and *anti* conformations.

Watson–Crick base pair with adenine residues Aa5 and Ab5, respectively. Fig. 2 shows the electron densities of these pairs. This result indicates 5-formyluracil can form a canonical Watson–Crick base pair with adenine in DNA in the same way as thymine.

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