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Crystallization and preliminary analysis of a DNA dodecamer of d(CGCGmo^{6}AATCCGCG) containing 2′-deoxy-N^6-methoxyadenosine: change in crystal packing with different humidity

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The DNA dodecamer of (CGCGmo^{6}AATCCGCG) containing 2′-deoxy-N^6-methoxyadenosine has been crystallized for X-ray analysis in order to investigate the effects of the modified adenosine on base pairing. It has been found that the crystal changes from one form to another during data collection in a manner similar to a phase transition. The two crystal structures show that this phenomenon, ascribed to differences in humidity, is correlated with a change in the contact angle between the two duplexes.

1. Introduction

Oxyamines such as hydroxyamine and methoxyamine are known mutagens which attack amino groups on the DNA bases (Singer & Kusmierek, 1982). In the case of an adenine derivative methoxylated at N(6), it has been shown that this modification allows the adenine base to tautomerize between amino and imino forms with the latter more stabilized under hydrophilic conditions (Stolarski *et al.*, 1984; Fujii *et al.*, 1987). Matsuda and his colleagues showed by an *in vitro* polymerase incorporation assay that when a DNA fragment containing the modified adenosine was used as a template, not only complementary thymidine residues but also cytidine residues were incorporated into the newly synthesized opposite DNA strand (Nishio *et al.*, 1992). It was also demonstrated that when the triphosphate of 2′-deoxy-N^6-methoxyadenosine derivatives was used as a reactant, it incorporated dATP and dGTP in a similar way (Hill *et al.*, 1998). The two crystal structures of the component N^6-methoxyadenine derivatives, N^6-methoxy-2′,3′,5′-tri-*O*-methyladenosine (Birnbaum *et al.*, 1984) and 9-benzyl-N^6-methoxyadenine (Fujii *et al.*, 1990), indicate that this modified base prefers an imino form (see Fig. 1) with the methoxyl group in the *syn* conformation to the N(1) atom around the C(6)—N(6) bond. In the *syn* conformation, however, the geometry of base pairing will be largely deformed from the regular Watson–Crick-type pairing, which might be a disadvantage during incorporation into a growing DNA strand. In the case of a methoxycytosine analogue, similar incorporation occurred during *in vitro* polymerization (Singer *et al.*, 1984; Reeves & Beattie, 1985). N^4-methoxycytidine also prefers an imino form in solution (Brown *et al.*, 1968; Morozov *et al.*, 1982). A DNA fragment containing this residue was examined by X-ray analysis (Meervelt *et al.*, 1990). The cytosine base takes an imino form as expected, but unfortunately the DNA conformation is Z-form and the cytosine moiety with the methoxyl group in the *syn* conformation forms a wobble base pair with guanine. To investigate base pairing upon methoxylation, the DNA molecule should have a B-form conformation. To date, no one has succeeded in preparing such a DNA molecule containing these methoxy-modified nucleotides. We have successfully incorporated methoxyadenosine into the DNA dodecamer d(CGCGmo^{6}AATCCGCG), chosen because the original sequence d(CGCGAATTCGCG) prefers a B-form conformation (Dickerson & Drew, 1981). In order to establish the tautomerization hypothesis, crystallographic studies of the derivative have been undertaken. During data collection, it was found that the DNA dodecamer crystal changes from one

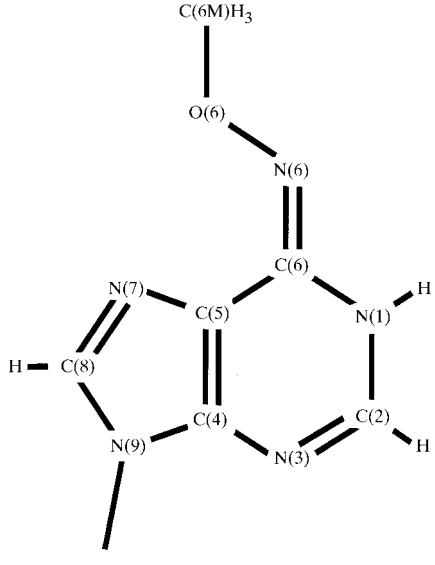

Figure 1
Structural formula of the N^6-methoxyadenosine base with individual atoms numbered.

Table 1
Crystallographic data and structure-determination statistics.

Crystal form	Form I	Form II
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell constants (Å)		
a	24.2	25.4
b	39.0	40.6
c	64.2	66.9
Z†	1	1
Solvent content‡ (%)	40.6	47.8
Limiting resolution (Å)	2.5	3.0
Observed reflections	9933	5795
Independent reflections	1692	1293
R_{merge} (%)	9.8	9.6
Completeness (%)	72.7 (30–2.5 Å resolution shell); 52.3 (2.8–2.5 Å resolution shell)	82.6 (30–3.0 Å resolution shell); 79.1 (3.5–3.0 Å resolution shell)
Non-H DNA atoms	488	488
Solvent molecules	21	7
Reflections used for refinement	1213	1192
Resolution range (Å)	7.5–2.8	10.0–3.0
R factor (%)	20.0	22.4
R_{free}§ (%)	25.7	28.6
R.m.s. standard deviations		
Bond lengths (Å)	0.010	0.011
Bond angles (°)	3.29	3.24
Improper angles (°)	1.79	1.59
Average coordinate error (Å)	0.35	0.45

† Number of duplexes in the asymmetric unit. ‡ Specific volume of the nucleic acid is assumed to be 0.74 $cm^3\ g^{-1}$. § Calculated using the 5% of reflection data which were not used for refinement.

crystal form to another. It is interesting that the conformation of such a short DNA oligomer can be changed by varying the humidity in the crystalline state. It is known that several conformations have been observed in fibrous DNA (Leslie *et al.*, 1980), and a change in cell dimensions with temperature has also been reported (Dickerson *et al.*, 1983). However, we are the first to report a transition which occurs with a change in humidity. In this paper, the crystal structures of the two forms have been solved and are compared.

2. Materials and methods

2′-Deoxy-N^6-methoxyadenosine was synthesized from 2′-deoxyadenosine according to the method of Nishio *et al.* (1992). It was then incorporated into the dodecamer d(CGCGmo^{6}AATCCGCG) at mo^{6}A on a DNA synthesizer. Crystallization conditions were surveyed in 10 m*M* sodium cacodylate buffer (pH 7) by changing the temperature and the concentrations of the DNA dodecamer, spermine tetrahydrochloride, magnesium acetate and 2-methyl-2,4-pentanediol (MPD) using the hanging-drop vapour-diffusion technique. The most suitable conditions were derived by a factorial analysis (Carter, 1992). Within two weeks, single crystals were obtained at 277 K from a 5 µl droplet containing 0.75 m*M* DNA dodecamer, 6 m*M* spermine tetrahydrochloride, 18 m*M* magnesium acetate and 10% MPD equilibrated against a 700 µl reservoir solution containing the same components (without DNA) at twice the concentration but at 35% for MPD. The crystals are hexagonal plates with dimensions of about 0.2 × 0.2 × 0.3 mm.

Initially, X-ray diffraction data were taken at 275 K by the oscillation method using a Rigaku R-AXIS IIc image-plate detector with Cu $K\alpha$ radiation generated by a rotating-anode generator operating at 50 kV and 80 mA. The crystal was rotated through a total of 180°. 45 frames were processed by the program *DENZO* (Otwinowski, 1993) to 2.5 Å resolution. As shown in Fig. 2, the unit-cell parameters were almost constant during the first half of data collection, but after the 26th frame they changed gradually and at the 32nd frame the *c*-axis length changed dramatically. This phenomenon suggests the existence of two crystalline forms (form I and form II). Therefore, the intensity data up to the 26th frame and after the 33rd frame were merged separately in the two data sets by *SCALA* and *AGROVATA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The 26 frames covered the independent reflections of form I with a data completeness of 93.2%. As the data completeness of form II was not very high, the intensity data were re-collected on the Sakabe–Weissenberg camera (Sakabe, 1991) using synchrotron radiation (λ = 1.00 Å) at the Photon Factory in Tsukuba. For reasons described later, another crystal was carefully mounted in a glass capillary with a small amount of the mother liquor to keep the form II crystal wet. The data collection was performed in an experimental hutch maintained at 275 K to prevent movement of water around the crystal. Diffraction spots recorded on imaging plates were indexed and their intensities integrated by the program *DENZO* (Otwinowski, 1993) to 3.0 Å resolution.

Initial phases of the two data sets (form I and form II) were derived by the molecular-replacement method with the program *AMoRe* (Navaza, 1994) using the atomic coordinates of the duplex dodecamer d(CGCGAATTCGCG)$_2$ (Dickerson & Drew, 1981) as a probe. The molecular structures were constructed and modified on a graphics workstation by referring to omit maps at every nucleotide residue with the program *O* (Jones *et al.*, 1991) and with the program *QUANTA* (Molecular Simulations Inc., San Diego, USA). The atomic parameters were refined with the program *X-PLOR* (Brünger, 1992). The final *R* factor was 20.0% for the form I data in the resolution range 7.5–2.8 Å (R_{free} = 25.7% for the 5% of reflection data which were not used throughout the refinement) and 22.4% for the form II data in the resolution range 10–3.0 Å (R_{free} = 28.6%). The crystallographic data and structure-determination statistics are given in Table 1.

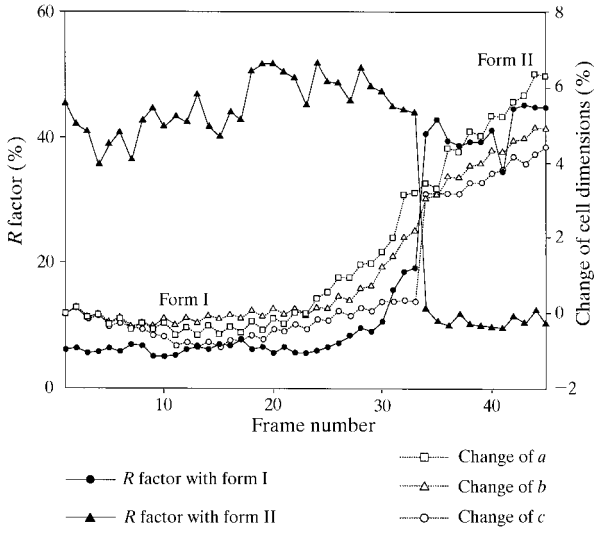

Figure 2
Change in the unit-cell dimensions of d(CGCGmo^{6}AATCCGCG) and of the diffraction intensities during data collection. The abscissa is the time course in exposed frame units. The right ordinate shows the changes in unit-cell dimensions from those of the first frame. The left ordinate is the *R* factor of intensities in each frame with each data set (form I or form II).

3. Results and discussion

The intensity data of each frame were compared for form I and form II. The *R* values in each frame are shown in Fig. 2 as a

function of the frame number instead of the time course. It is noted that the R values changed suddenly between frames 32 and 33. This behaviour suggests that the crystal structure changed in a similar manner to a phase transition between the two crystal forms, although no change in space group occurred. Nevertheless, the unit-cell parameters enlarge gradually in frames 26–31 and more so from the 33rd frame to the final one. At the 32nd frame, however, the c axis lengthens dramatically. It is interesting to consider the reason why this change occurs during the experiment. After the data collection, it was observed that the crystal mounted in the capillary was bathed in liquid, although prior to the data collection the crystal had been mounted with no liquid around it. The water molecules from the mother liquor sealed in the bottom of the capillary had condensed at the crystal, which was at lower temperature than the rest of the capillary, as only the crystal was cooled to 275 K. It is plausible that the intact crystal picked up from the mother liquor changed from form II to form I as a consequnce of the removal of water from around the crystal in the capillary. Therefore, the crystal parameters might fluctuate during a change in humidity. In fact, the second crystal mounted with a small amount of the mother liquor remained in form II throughout data collection.

The two crystal structures have been compared. The two chains of the DNA dodecamer form a duplex with a B-form conformation in both crystal forms. There are no large changes in the DNA conformation between the crystal forms, as was observed in DNA fibres at different humidities (Leslie *et al.*, 1980). The detailed molecular structures are almost the same, though slight local differences occur. In both crystal forms, the DNA duplexes make contact with other duplexes related by 2_1 symmetry along the c axis at both ends by forming two extra base pairings, with two N(2)—H···N(3) hydrogen bonds between guanine moieties on different helices. This packing motif is similar to that reported for the $d(CGCGAATTCGCG)_2$ crystal (Dickerson & Drew, 1981; Shui *et al.*, 1998). The only significant difference is found in the contact angle between the two duplexes. As seen in Fig. 3(*a*), the angle between the two helix axes is more open in form II by 12°. This opening of the angle lengthens the c axis by 4%. The resulting c-axis length is in agreement with the real value observed in the form II crystal. The conclusion from the above results is that the observed transition between the two forms arises from a packing change caused by water molecules incorporated into the crystal. Unfortunately, at the present resolution it is difficult to analyse the hydration structure surrounding the DNA dodecamers.

The original purpose of the present investigation was to reveal the hydrogen-bonding scheme between the N^6-methoxyadenine and the cytosine moieties. Although the resolution is rather low, electron-density maps (Fig. 3*b*) suggest that the modified adenine has a methoxyl group with an *anti* conformation to the N(1) atom around the C(6)—N(6) bond, and forms a base pair with a cytosine base on the complementary chain in a manner identical to Watson–Crick-type pairing. To form these types of hydrogen bonds, the modified adenine base must take an imino form. This is the first example exhibiting a mispairing of an adenine base induced by methoxylation. More recently, X-ray data from the form II crystal have been obtained at 110 K using synchrotron radiation. The crystal diffracted to 1.6 Å resolution. A more detailed structure analysis including water molecules will be reported in the future.

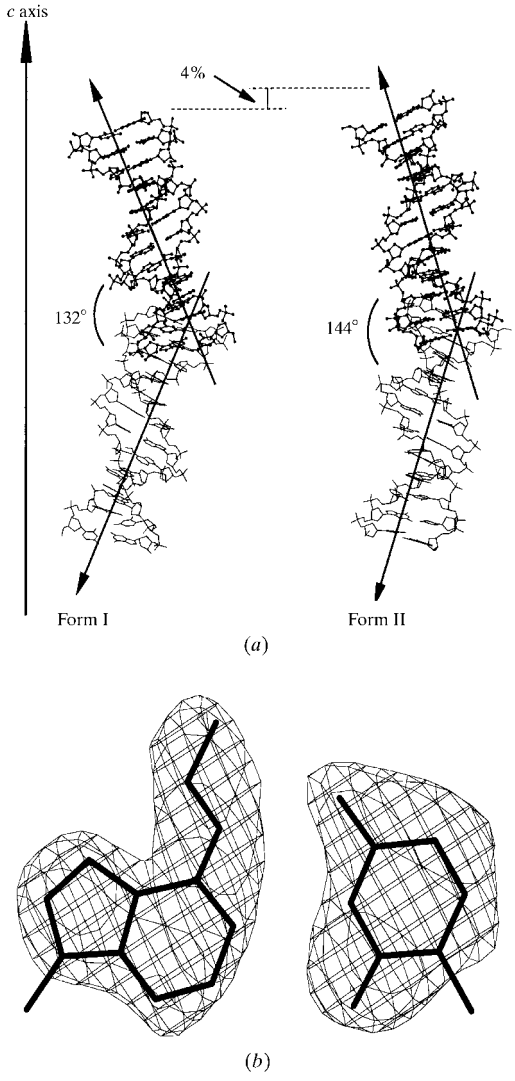

Figure 3
(*a*) The contact angle between the two duplexes. The difference between the two crystal forms explains the c-axis expansion in form II. (*b*) An omit $|F_o| - |F_c|$ map of an N^6-methoxyadenine moiety paired with a cytosine moiety on the opposite strand, contoured at the 2.5σ level.

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