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Crystallization and preliminary X-ray analysis of the DNA decamers d(CCGGATCCGG) and d(CCGGCGCCGG)

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The DNA decamers d(CCGGATCCGG) and d(CCGGCGCCGG) have been crystallized for X-ray analysis in order to investigate the effects of changing the two central base pairs of the DNA fragment d(CCGGGACCGG). Previous studies have already demonstrated that the structure of the former DNA fragment contains a DNA Holliday junction. Crystals were obtained at 293 K by the hanging-drop vapour-diffusion technique using the Nucleic Acid Mini Screen. Over a period of two weeks, hexagonal plates appeared. For the DNA fragment d(CCGGATCCGG), the crystals belong to space group $P3_1$, with unit-cell parameters $a = b = 33.54$, $c = 46.39$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$, and diffract to 2.2 Å. In the case of the DNA fragment d(CCGGCGCCGG) the crystals belong to the space group $C2$, with unit-cell parameters $a = 65.35$, $b = 24.07$, $c = 37.34$ Å, $\beta = 109.97^\circ$, and diffract to 2.0 Å.

1. Introduction

Following the solution of the structure of the DNA decamer d(CCGGGACCGG), which contains a four-way DNA junction (Ortiz-Lombardia *et al.*, 1999), it was decided to investigate the related fragments d(CCGGATCCGG) and d(CCGGCGCCGG), which have the two central base pairs in the usual Watson–Crick linkage.

The four-way DNA junction is an important structural intermediate in recombination, viral integration and DNA repair where the DNA double helix is disrupted. It was first proposed almost 40 y ago (Holliday, 1964) and is also termed the Holliday junction. Since then, a large amount of data have been obtained by gel electrophoresis (Duckett *et al.*, 1988), fluorescence resonance energy transfer (Miick *et al.*, 1997), NMR (Overmans & Altona, 1997), molecular modelling (von Kitzing *et al.*, 1990; Wood *et al.*, 1997) and enzyme or chemical probing (Grainger *et al.*, 1998).

Recently, we have solved the structure of the first DNA four-way junction in the DNA fragment d(CCGGGACCGG) (Ortiz-Lombardia *et al.*, 1999), which is formed by two continuous B-DNA helices running anti-parallel and crossing in a right-handed sense. All the bases are included in the crossing B-DNA helices and the exchanging backbone is formed only of sugars and phosphates with strong van der Waals contacts and hydrogen bonds.

The two DNA cylinders which comprise the structure are in the B form with very few structural changes. The two G·A mismatches are not at the centre of the Holliday junction and do not interfere with the crossing of the arms. A similar observation has been made in the structure of a DNA fragment with the sequence d(CCGGTACCGG) (Eichman *et al.*, 2000). The crystal structure of a Holliday junction in a DNA–RNA complex has been reported previously (Nowakowski *et al.*, 1999); in this case, the DNA/RNA are in the A form.

2. Methods and results

Several crystallization trials were made using the Nucleic Acid Mini Screen solutions (Berger *et al.*, 1996). Crystals were grown at 293 K by the hanging-drop vapour-diffusion technique using Linbro multiwell tissue-culture plates. Crystals for the first fragment grew in a drop containing 20 m*M* sodium cacodylate pH 6, 5% MPD, 6 m*M* spermine, 40 m*M* KCl, 10 m*M* $MgCl_2$ and 1.5 m*M*

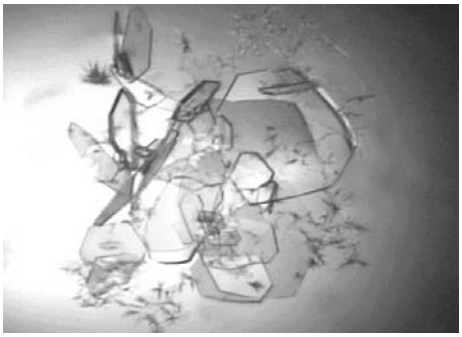

Figure 1
Crystals of the DNA decamer d(CCGGATCCGG).

Table 1
Summary of the d(CCGGATCCGG) and d(CCGGCGCCGG) data-collection statistics obtained at cryogenic temperatures.

Values for the last shell [2.32–2.20 Å for d(CCGGATCCGG) and 2.15–2.00 Å for d(CCGGCGCCGG)] are given in parentheses.

	d(CCGGATCCGG)	d(CCGGCGCCGG)
Unit-cell parameters (Å, °)	$a = b = 33.54$, $c = 46.39$, $\alpha = \beta = 90$, $\gamma = 120$	$a = 65.35$, $b = 24.07$, $c = 37.34$, $\alpha = 90$, $\beta = 109.97$, $\gamma = 90$
Space group	$P3_1$	$C2$
Data resolution (Å)	2.2	2.0
No. of unique data	2919	3623
Completeness (%)	98.5 (98.5)	94.4 (83.1)
Multiplicity	2.3 (2.4)	2.2 (2.0)
$R_{\rm merge}$† (%)	9.7 (48.2)	4.8 (41.8)
Mean $\langle I/\sigma(I)\rangle$	2.2 (1.1)	17.6 (1.7)

† $R_{\rm merge}(I) = \sum_h \sum_i |I_i - I| / \sum_h \sum_i I$, where I is the mean intensity of i reflections.

d(CCGGATCCGG). After two weeks hexagonal plates appeared (Fig. 1). Crystals were flash-frozen in a stream of evaporating nitrogen at 120 K. Diffraction data were collected at the ESRF (Grenoble, France) beamline ID14 to 2.0 Å using a wavelength of 0.95 Å (see Table 1). Data processing was performed with *MOSFLM* (Leslie, 1991) and data were scaled, merged and reduced with programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The crystal had dimensions of 0.1 × 0.3 × 0.4 mm and belonged to space group $P3_1$. The structure determination of d(CCGGATCCGG) is under way.

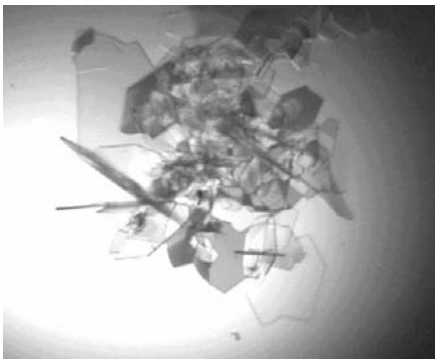

Figure 2
Crystals of the DNA decamer d(CCGGCGCCGG).

Crystals for the second DNA fragment appeared in a drop containing 20 m*M* sodium cacodylate pH 6, 6.7% MPD, 8 m*M* spermine, 53 m*M* NaCl, 8 m*M* KCl, 6.7 m*M* $MgCl_2$ and 1 m*M* d(CCGGCGCCGG). Thin plate-like crystals appeared in three weeks (Fig. 2). Data were collected at the EMBL Outstation beamline BW7A (DESY, Hamburg) to 2.0 Å using a wavelength of 1.1 Å (see Table 1). Data were processed with *DENZO* and reduced with *SCALEPACK* (Otwinowski & Minor, 1997). The unit-cell parameters of the crystal of this DNA decamer are similar to the original fragment d(CCGGGACCGG) in which the first DNA four-way junction was found (Ortiz-Lombardia *et al.*, 1999).

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