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A preliminary neutron crystallographic study of an A-DNA crystal

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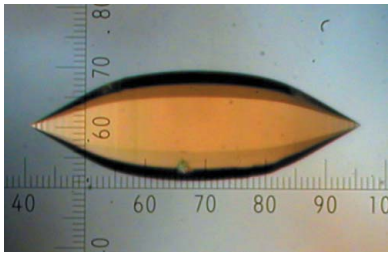
The LADI-III diffractometer at the Institut Laue–Langevin has been used to carry out a preliminary neutron crystallographic study of the self-complementary DNA oligonucleotide d(AGGGGCCCCT)$_2$ in the A conformation. The results demonstrate the viability of a full neutron crystallographic analysis with the aim of providing enhanced information on the ion–water networks that are known to be important in stabilizing A-DNA. This is the first account of a single-crystal neutron diffraction study of A-DNA. The study was carried out with the smallest crystal used to date for a neutron crystallographic study of a biological macromolecule.

1. Introduction

DNA is capable of extensive structural polymorphism (Fuller *et al.*, 2004). The role of water and ions in stabilizing DNA conformations and in mediating structural transitions between them is well known. Natural (mixed-sequence) DNA can adopt the A, B and C conformations depending on hydration and the prevailing conditions of ionic strength. A number of repetitive-sequence DNAs that may be of biological importance are capable of adopting other conformations such as the left-handed Z conformation and the tightly wound D conformation. Water-mediated transitions between these structures can be followed in real time using synchrotron X-ray sources (Mahendrasingam *et al.*, 1986; Forsyth *et al.*, 1986) and crystallographic approaches have been used to trap DNA oligonucleotides in a range of structures that relate to the A–B structural change (Vargason *et al.*, 2001).

In the continuous polymer, A-DNA is an 11-fold right-handed double helix having a deep major groove and heavily tilted bases that are displaced away from the axis towards the minor groove. The molecule has an open centre of approximately 6 Å diameter around the axis. This type of structure is adopted by RNA double helices (see, for example, Shi *et al.*, 1999) and DNA–RNA hybrid molecules (Fedoroff *et al.*, 1993; Xiong & Sundaralingam, 2000) and there has been speculation that the A conformation is involved in transcription. In naturally occurring DNA and throughout a large range of synthetic DNA polymers, the A conformation is capable of structural transitions to the C and the B conformations. A-type conformations are also found in oligonucleotide–protein complexes (Kim, Nikolov *et al.*, 1993; Kim, Geiger *et al.*, 1993) and are known to bind to a number of anticancer antibiotics (*e.g.* Welch *et al.*, 1994).

A fair amount of information on DNA hydration is available from X-ray crystallography (Egli *et al.*, 1998; Kennard *et al.*, 1986; Malinina *et al.*, 1998), neutron fibre diffraction (Forsyth *et al.*, 1989; Langan *et al.*, 1992; Shotton *et al.*, 1997) and, more recently, neutron crystallography (Arai *et al.*, 2005; Chatake *et al.*, 2005). These approaches are highly complementary in the information that they provide. High-resolution X-ray methods have provided considerable detail on the location of structured water in oligonucleotide duplexes. However, it is frequently difficult to use X-ray diffraction methods to extract complete information on the location of water and H atoms, parti-



cularly for atoms that have high thermal displacement parameters (Tereshko *et al.*, 2001). High-angle neutron fibre-diffraction experiments that exploit the difference in coherent scattering of H_2O and D_2O provide unique information that relates to the continuous polymer, in a situation where crystal packing effects are minimized but where the resolution of the observed data is typically poorer than that obtained for single-crystal studies. More recently, neutron Laue methods have been developed at both steady-state (Blakeley, Ruiz *et al.*, 2008) and spallation (Langan & Greene, 2004) neutron sources. Since neutron diffraction studies of oligonucleotide single crystals are sensitive to the location of H atoms, they may provide additional detail on the orientation of water molecules and hence on the nature of hydrogen-bond interactions.

The oligonucleotide sequence d(ApGpGpGpGpCpCpCpCpT) is of interest because of its potential significance for understanding the marked stability of A-type DNA structures in guanine–cytosine homopolymer repeats such as poly-d(G)·poly-d(C). It is known to crystallize in two different space groups, $P2_12_12_1$ and $P6_122$, and previous work on similar sequences (Gao *et al.*, 1995) has demonstrated that while there is a clear influence of the packing on the molecular conformation, the main features of A-DNA are maintained in both symmetries. X-ray structures of the $P2_12_12_1$ (at 1.1 Å resolution) and $P6_122$ (at 1.5 Å resolution) forms of d(AGGGGCCCCT)$_2$ have been described by Gao *et al.* (1999). Here, we describe preliminary results from the first neutron crystallographic study of an A-type conformation of an oligonucleotide. The study builds on a growing body of results from the LADI-III diffractometer at the Institut Laue–Langevin (ILL) (Blakeley, 2009; Teixeira, Zaccai *et al.*, 2008).

2. Methods

2.1. Crystallization

HPSF grade (High Purity Salt Free) oligonucleotides were obtained from MWG Biotech. The oligonucleotides were further purified by cation-exchange chromatography (Pharmacia MonoQ HR 10/10), dialysis and a final desalting step using a Biorad EconoPac column. The oligonucleotides were flash-frozen and lyophilized prior to solubilization in water to obtain the final stock concentration. The concentration of the samples was determined by UV spectroscopy. The samples were annealed from 368 K prior to crystallization.

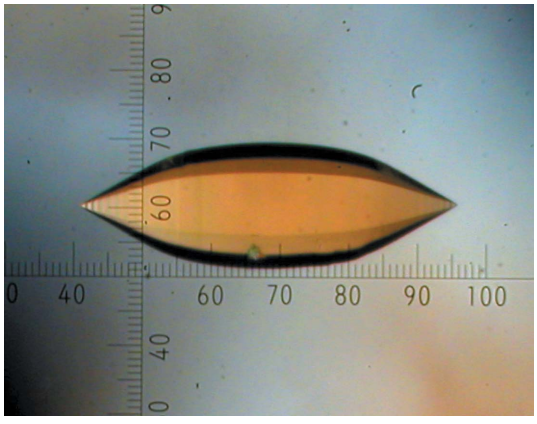


Figure 1
The oligonucleotide crystal used for this neutron diffraction study on LADI-III. The crystal volume was estimated to be 0.06 mm^3.

Crystals were obtained at room temperature in hydrogenated buffer using the sitting-drop vapour-diffusion method. Crystallization conditions were optimized from the previously published protocol (Gao *et al.*, 1999) to obtain crystals that grew in a few days in 10–40 µl drops containing a 1:1 mixture of DNA and 40 m*M* sodium cacodylate buffer pH 6, 10 m*M* cobalt hexammine, 120 m*M* magnesium chloride, 80 m*M* lithium chloride, 10% 2-methyl-2,4-ethylpentanediol (MPD). The drops were equilibrated against a reservoir consisting of 35% MPD. The DNA concentration was optimized at 2 m*M*, which systematically yielded a smaller number of larger crystals. In order to further optimize the growth of large crystals, vapour diffusion was limited by the application of a layer of Al's oil (a 1:1 mixture of parafin and silicone oil) onto the surface of the MPD in the reservoir (Chayen *et al.*, 1997). This compensated for the different diffusion rates that were particularly obvious at drop volumes above 30 µl and where the incidence of multiple crystals was otherwise higher. Approximately four weeks prior to data collection, the crystals were mounted on quartz capillaries and left to equilibrate by vapour diffusion against deuterated crystallization buffer to reduce neutron incoherent scattering from exchangeable hydrogen in the crystal sample.

2.2. Neutron diffraction

Neutron Laue diffraction data were recorded at room temperature using the LADI-III diffractometer at the ILL. The LADI-III instrument, which uses a large neutron image-plate detector that completely encircles the sample, is a recent replacement for the LADI-I instrument that was used to collect data for human aldose reductase (Blakeley *et al.*, 2006; Blakeley, Ruiz *et al.*, 2008), xylose isomerase (Meilleur *et al.*, 2006), concanavalin A (Blakeley *et al.*, 2004; Ahmed *et al.*, 2007), rasburicase (Budayova-Spano *et al.*, 2006) and endothiapepsin/hydroxyethylene (Coates *et al.*, 2006). More recently, the instrument has been used to study the hydration and protonation states of thaumatin (Teixeira, Blakeley *et al.*, 2008). The improved design of LADI-III provides a twofold to threefold gain in neutron detection (Wilkinson *et al.*, 2007) and the larger radius of the instrument aids the signal-to-noise ratio of recorded reflections and decreases spatial overlap.

A nickel/titanium multi-layer bandpass filter was used to select a neutron wavelength range ($\Delta\lambda/\lambda \simeq 25\%$) extending from 3.1 to 4.2 Å and centred at 3.7 Å. Data were recorded in a series of 18 Laue images with a step separation of 5° about the vertical rotation (φ) axis of the detector. Neutron Laue data were indexed and integrated using the *LAUEGEN* software suite (Helliwell *et al.*, 1989; Campbell, 1995) modified for the cylindrical geometry of the LADI-III detector (Campbell *et al.*, 1998). The *LSCALE* program (Arzt *et al.*, 1999) was used to derive the wavelength-normalization curve using the intensities of symmetry-equivalent reflections measured at different wavelengths. *SCALA* (Collaborative Computational Project, Number 4, 1994) was used to combine and merge the observed data. There was no appreciable radiation damage to the sample and no explicit absorption correction was applied.

2.3. X-ray diffraction

Two X-ray data sets were collected in connection with this work. Since an important goal of this study was to be able to carry out a joint X-ray/neutron refinement, room-temperature data were collected at medium resolution from the same crystal as used for the neutron data collection. This was performed with an in-house diffractometer using Cu $K\alpha$ radiation (λ = 1.5418 Å) equipped with a MAR345 image-plate detector. The data were integrated and

reduced using *MOSFLM* (Leslie, 1992) and *SCALA* and *TRUNCATE* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). Observations were rejected during scaling if they deviated by more than five standard deviations from the mean. Immediately after the room-temperature data had been recorded, the crystal was cryocooled in a goniometer loop and data to a resolution of 1.3 Å were recorded on beamline ID23-1 at the ESRF.

3. Results

Fig. 1 shows a photograph of the crystal used for the preliminary neutron work described here. The crystal was 1.12 mm long and 0.36 mm wide and was hexagonally symmetric about its long axis. It was mounted in a thin-walled quartz capillary. The volume of the crystal was calculated to be around 0.06 mm^3. Fig. 2 shows a representative example of the neutron Laue diffraction patterns recorded from this sample. Table 1 shows the relevant neutron and (room-temperature) X-ray data-collection statistics. There were 6724 observed reflections recorded for the neutron data collection. Interestingly, preliminary density maps based on phases derived from the published X-ray model suggest the presence of D atoms at some of the guanine 7 positions (data not shown).

4. Discussion

The final stages of data collection are currently under way; this will involve further data collection to improve resolution and completeness and will be used to pursue a full analysis of $d(AGGGGCCCCT)_2$ in the A conformation with the aim of providing detailed structural characterization inclusive of hydration and protonation. This analysis will exploit the availability of both neutron and X-ray data sets from the same sample and a joint structure refinement will be carried out using the *nCNS* (Langan & Mustyakimov, 2009) and *PHENIX* (Afonine *et al.*, 2005) programs. The results will provide further information on the factors that stabilize this type of DNA conformation.

This study highlights a number of technical issues that are of general interest for neutron crystallography. Firstly, the work was carried out using a crystal that is the smallest ever described to date for a neutron crystallographic study of a biological macromolecule. In making such comparisons, one should of course consider the ratio between the sample volume and the volume of the asymmetric unit (Blum *et al.*, 2007; Helliwell, 1992), which in this case is 49 (Blakeley, Langan *et al.*, 2008) and is the third smallest ratio to date after the study of aldose reductase by Hazemann *et al.* (2005) and that of dihydrofolate reductase (Bennett *et al.*, 2006). This is a tribute to recent developments at the LADI-III diffractometer at the ILL and emphasizes the major gains possible in the scope of neutron macromolecular crystallography as this instrument is optimized. Current instrument developments, together with the imminent move of LADI-III to a site that will provide a major flux gain, are likely to yield a future efficiency gain of ∼10. Secondly, while in this experiment hydrogen incoherent scattering was substantially reduced by the use of a crystal containing deuterated buffer, the oligonucleotide itself was not perdeuterated and a large amount of hydrogen remains attached to the C atoms of the furanose sugar groups and the bases. Further major gains in terms of the minimum usable sample volume can be expected with the use of perdeuterated oligonucleotide samples. In neutron protein crystallography, perdeuteration is usually associated with an effective gain factor of ∼10 in the minimum required crystal volume. Given the crystal volumes typically attainable in oligonucleotide or oligonucleotide–ligand systems, such a gain, in tandem with the developments planned for LADI-III, would dramatically widen the scope of nucleic acid crystallography. Thirdly, this type of work on nucleic acids is likely to be complemented in the future by studies in which other neutron and X-ray scattering techniques are brought to bear on situations in which crystallization is not possible or where parametric studies are envisaged. For example, Miles *et al.* (2009) have recently characterized using small-angle scattering the variation of DNA quadruplex structure in response to changes in ionic strength and temperature. This work has also illustrated how selective deuteration of DNA oligonucleotides could be heavily exploited.

Table 1
Data-collection statistics for the oligonucleotide crystal.

Values in parentheses are for the highest resolution shell.

Data set	Neutron	X-ray
Beamline	LADI-III	Rotating anode
Wavelength (Å)	3.1–4.2	1.54
No. of images	18	89
Oscillation angle (°)	5	1
Exposure time per image (min)	720	1
Space group	$P6_122$	
Unit-cell parameters (Å)	$a = b = 33.021$, $c = 78.817$	
Resolution range (Å)	26.88–2.30 (2.42–2.30)	26.88–1.92 (2.02–1.92)
No. of unique reflections	1082 (119)	2218 (265)
Completeness (%)	82.4 (66.6)	97.7 (84.5)
R_{merge}†	0.18 (0.25)	0.043 (0.383)
Multiplicity	6.2 (2.8)	8.7 (7.3)
Mean $I/\sigma(I)$	11.8 (2.1)	19.3 (4.1)
Wilson B factor ($Å^2$)	32.1	36.0

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith measurement of reflection hkl and $\langle I(hkl)\rangle$ is the mean value of $I(hkl)$ for all i measurements.

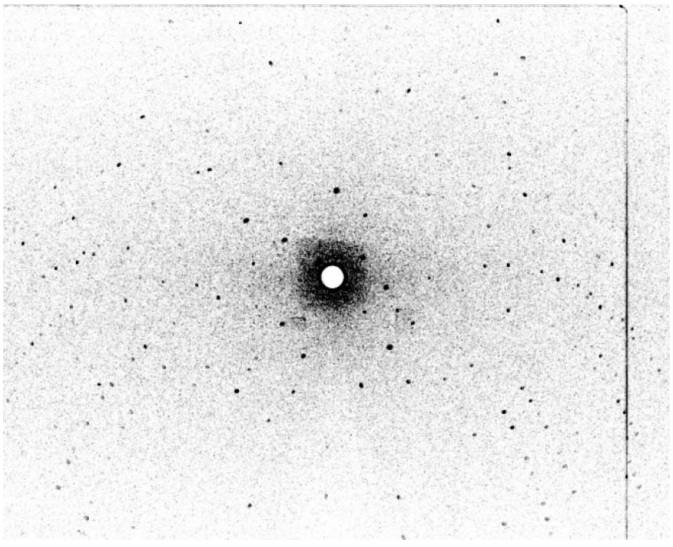


Figure 2
Neutron Laue diffraction pattern recorded from the hydrogenated oligonucleotide crystal shown in Fig. 1 using the LADI-III instrument at the ILL. The exposure time was 12 h.

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