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A high-angle neutron fibre diffraction study of the hydration of deuterated A-DNA

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Abstract

A high-angle neutron fibre diffraction study of the hydration of A-DNA has been performed using the single-crystal diffractometer D19 at the Institut Laue-Langevin (Grenoble, France). The sample was prepared using deuterated DNA extracted from *E. Coli* cells cultured on deuterated nutrients. In common with our previous neutron fibre diffraction studies of DNA, this work exploits the ability to isotopically replace H_2O around the DNA by D_2O. However this study benefitted additionally from the fact that the hydrogen atoms which are covalently bonded to carbon atoms in the DNA sugars and bases were replaced by deuterium so that incoherent scattering and absorption effects were minimised. Successive cycles of Fourier synthesis and Fourier difference synthesis allowed water peaks to be identified and their positional and occupancy parameters to be refined against the observed diffraction data. The results confirm the main hydration features noted in our earlier studies with a clear network of water running along the inside edge of the major groove linking successive O1 phosphate oxygen atoms. The central core of water running along the axis of the double helix is very much clearer in this work. Additionally this study shows chains of ordered water lying in the centre of the major groove. © 1997 Elsevier Science B.V.

Keywords: DNA; DNA hydration; Neutron high-angle fibre diffraction; Isotopic replacement; Deuteration

1. Introduction

The deoxyribonucleic acid (DNA) double helix can adopt one of five major variants depending on base-pair sequence, hydration and the nature of its ionic environment. The A, B, and C conformations were first identified in natural DNA but are also observed routinely in a wide range of synthetic polynucleotides. Two-stranded synthetic DNA polymers having regular repetitive base-pair sequences can in addition adopt a number of variations of these conformations and also novel conformations such as the D conformation which is adopted by poly[d(A-T)] · poly[d(A-T)] and the left-handed S conforma-

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tion which has been most commonly reported for the poly[d(G-C)] · poly[d(G-C)] double helix. Of these five principal conformations of DNA, the A [1], B [2], C [3] and D [4] conformations were all first determined by X-ray fibre diffraction but the left-handed S conformation was first discovered as the Z conformation in an X-ray single-crystal analysis of an oligonucleotide [5] and then subsequently reported in X-ray fibre diffraction studies where it was designated S-DNA [6]. Fibre diffraction studies of long polymeric DNA, single-crystal studies of short oligonucleotides, and computer modelling studies have all provided evidence that water and cations in these structures occupy well-defined locations which are crucial to the stabilisation of the various conformations that can be adopted by the DNA. Further evidence for this has come from the observation of structural transitions between these different double helical conformations in DNA fibres. Several humidity-driven conformational transitions have been studied using time-resolved high-angle X-ray fibre diffraction at the Daresbury Laboratory synchrotron radiation source [7–9].

The A-conformation of DNA is an 11-fold right-handed double helix which crystallises in the monoclinic space group $C2$. Base pairs are tilted by approximately 20° relative to the helix axis and displaced away from the axis towards the minor groove. This creates an open core of approximately 6 Å in diameter around the axis, a deep major groove and a shallow minor groove. Speculation that the A conformation may be involved in the process of transcription has persisted since it was first observed that this type of structure and close variants of it are adopted not only by DNA but also by RNA double helices and DNA–RNA hybrids. In naturally occurring DNA and throughout a large range of synthetic polymers, the A conformation is capable of undergoing reversible transitions to the C and the B conformations.

Single-crystal studies of A type oligomers have revealed extensive hydration of phosphate oxygen atoms and bases. A number of authors have presented evidence for intrastrand water bridges linking successive phosphate oxygen atoms [10,11]. However it is also worth noting that some single-crystal studies have found that the phosphates in A type oligomers are individually hydrated [12,13]. In a study of the A-DNA fragment d(CpCpGpG), a network of solvent molecules was seen to stretch across the opening of the major groove, connecting phosphates on opposite sides of the groove [14]. In addition, water molecules arranged in fused pentagons, fixed by hydrogen bonding to base and phosphate oxygen atoms, were observed in the major groove of the TATA and ^{Br}UA ^{Br}UA sequences in studies of two A-DNA octamers [10].

While single-crystal studies of oligonucleotides offer important advantages over fibre diffraction studies, particularly in terms of resolution, care must be taken in extrapolating the results obtained from short oligonucleotides to the extended polymer, since end effects, crystal packing forces and the presence of crystallising agents may all play a part in determining the structure of these oligomers. Fibre diffraction experiments are typically more limited in resolution but provide information which clearly relates to the regularity of the extended polymer. The crystallite length along the fibre axis can be estimated from the profiles of the diffraction peaks and is typically ~ 150 Å corresponding to approximately five turns of the A-DNA double-helix (not taking into account the broadening effects of the beam width which would significantly increase this estimate). Furthermore the actual length of the DNA molecules making up the polycrystalline sample is of the order of 10^4 base pairs so that a single molecule can be expected to pass through many crystallites. Although X-ray fibre diffraction is a very powerful technique for the study of DNA conformation and for the study of conformational transitions, it is less well suited to the study of water around DNA. For this reason, neutron high-angle fibre diffraction was developed as a method that proved to be better suited to the investigation of the location of water around polymeric DNA [15]. The ability to isotopically replace H_2O in the DNA sample by D_2O allows the large coherent scattering length of deuterium to be utilised in imaging the water distribution around DNA. Neutron beam sources however have the disadvantage of relatively low flux, with the consequent requirement for large samples and counting times. The first application of neutron high-angle fibre diffraction was in the location of water around the D conformation of the double helix using instrument D19 at the Institut Laue-Langevin [15,16]. In subse-

quent work, an analogous study of the A conformation of naturally occurring hydrogenated DNA was undertaken [17,18]. The main features of this work were an extremely pronounced string of water molecules running along the inside of the major groove, linking successive O1 phosphate oxygens on the same strand, and a central core of water running down the axis of the helix. A theoretical study of A-DNA hydration has also identified these features and is described by Garcia et al. [19].

One of the major problems encountered in neutron fibre diffraction studies of DNA arises from the presence of large amounts of hydrogen in the sample. In DNA there are 20 non–exchangeable covalently bonded hydrogen atoms (i.e., those attached to carbon atoms) for an A-T nucleotide base pair, and 17 for a G-C nucleotide base pair. In addition, density measurements of A-DNA have indicated the presence of nine water molecules per nucleotide. Hydrogen has significantly larger incoherent and total neutron scattering cross sections than the other elements in DNA. Therefore its presence leads to a high level of incoherent background scattering in the recorded diffraction pattern and an increase in the effective absorption of diffracted neutrons by the sample. Both of these effects are highly undesirable in neutron diffraction experiments as they lead to reduced peak height to background ratios for Bragg reflections. In this study, these effects were minimised by preparing the sample from deuterated DNA obtained from *E. Coli* cells cultured in deuterated media and by the substitution of D_2O for H_2O in the sample environment.

2. Experimental methods

2.1. Sample preparation and environment

DNA was obtained from MRE600 *E. Coli* cells grown in 95% D_2O, using deuterated succinate as a carbon source. The cells were cultured during the course of an EMBO fellowship held at the EMBL Outstation in Grenoble. Deuterated double-stranded DNA was purified from these cells using standard techniques during which the cells were lysed, treated with proteinase K, and then subjected to several cycles of phenol and chloroform treatment. After the bulk of the protein had been removed, the DNA was precipitated with ethanol, washed, and then treated with RNAse and S1 nuclease. This was followed by another cycle of phenol/chloroform treatment which served to remove these enzymes and other residual proteins. The DNA was then dialysed against 10 mM sodium chloride and concentrated gels prepared by centrifugation at 50,000 rpm for 12 h. Individual fibres (approximately 5 mm long and 0.1 mm in diameter) were drawn from this gel using standard techniques [20]. X-ray diffraction tests showed that the deuterated A-DNA produced an identical diffraction pattern to that of hydrogenated A-DNA. In order to produce a sample of the required volume for neutron fibre diffraction studies, approximately 100 of these fibres were arranged into a parallel array that was tightly constrained by thin perforated aluminium foil.

The sample was mounted on a goniometer and housed within a sample enclosure having thin aluminium foil windows to allow the passage of incident and diffracted neutrons. The relative humidity of the sample environment and hence the D_2O content of the sample was regulated by the passage of humidified helium gas through the sample container. The relative humidity of the helium gas was maintained at a value of 75% by bubbling it through a saturated solution of sodium chlorate in D_2O, and monitored using a Vaisala HMP 31UT humidity probe located within the sample container. Previous X-ray diffraction studies of individual fibres from the sample had shown that the crystallinity of the sample was optimal in these conditions.

2.2. Data collection

The D19 diffractometer at the ILL has been described [21]. In the current work this instrument was used with a monochromatic beam of neutrons of wavelength 2.417 Å, produced by Bragg reflection from the (002) plane of a focusing graphite monochromator which was curved about its vertical axis. The wavelength spread $\Delta\lambda/\lambda$ was $\sim 4\%$ and the $\lambda/2$ harmonic was reduced to about 1% by graphite filters placed in the incident beam path. The beam size was limited by adjustable rectangular lithium fluoride slits. The detector was a banana-shaped multiwire gas-filled position sensitive device with a

vertical aperture of 64° and a horizontal aperture of 4°. The long axis of the detector had a radius of curvature of 1.164 m and was symmetrically located about the equatorial plane with its long axis vertical. The detector had 16 cathodes parallel to its long axis with a 5.0 mm wire spacing and 512 anodes parallel to its short axis with a spacing of 2.54 mm, giving an angular resolution of $0.25° \times 0.125°$. The sample was positioned at the centre of curvature of the detector. The detector was calibrated for nonuniformity of response by recording the diffuse scattering from a 1 cm diameter solid cylindrical rod of vanadium. An online data acquisition system (MAD) [22] enabled diffractometer movements and data collection to proceed according to a preselected set of instructions.

The application of novel data collection strategies for fibre diffraction experiments on this instrument has been described by Langan et al. [23]. The data collection strategy involved recording the entire diffraction pattern from the sample as a series of 4° 'strips'. A 1° overlap between successive strips allowed the outer cathodes on the detector which suffer from edge effects to be discarded. The recorded diffraction pattern consisted of 19 strips merged together and provided a resolution of approximately 3 Å. The sample was held at a fixed orientation relative to the incident beam during the collection of data.

2.3. Data reduction

Data display and initial processing were carried out using the suite of software available on the Silicon Graphics Indy at the D19 station. This software was accessed via a graphical user interface, McFibre, written in Metacard Hypertext [24]. A correction for the effective absorption of diffracted neutrons by the sample was applied using the absorption and scattering cross sections from the compilation of Koester et al. [25]. The corrected diffraction data were then binned into reciprocal space and the lattice

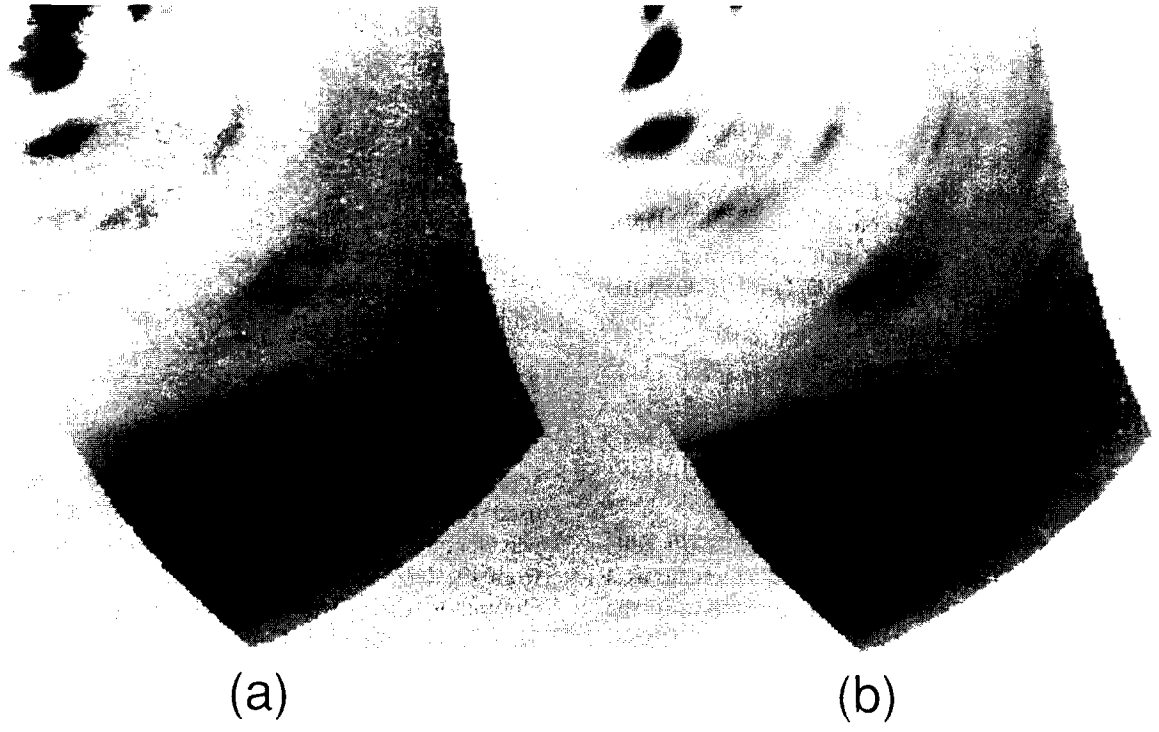

Fig. 1. (a) Diffraction data recorded from deuterated A-DNA hydrated with D_2O. The data has been binned into cylindrical $r-z$ space; (b) LSQINT fit of the data. Some data close to the meridian was not collected, as can be seen from the 'blind regions' in these two figures. This was due to physical constraints in mounting the sample.

parameters determined using purpose-designed software. The integrated intensities of the Bragg reflections were measured using the CCP13 routine LSQINT [26]. This routine allowed the background scattering to be fitted and subtracted from the pattern before the Bragg reflections were fitted with Lorentzian profiles using a maximum entropy method.

3. Results

Fig. 1a shows the recorded diffraction data binned into reciprocal space. The unit cell was monoclinic with lattice parameters $a = 22.24$ Å, $b = 40.62$ Å, $c = 28.15$ Å, $\beta = 97.0°$, in close agreement with the parameters published for X-ray fibre diffraction work [1]. Fig. 1b shows the LSQINT fit to the data. The intensities produced by LSQINT were used to compute a list of observed amplitudes for use in subsequent Fourier map generation and positional/occupancy refinement. In space group $C2$, systematically related reflections which overlap exactly as a result of cylindrical averaging of the fibre diffraction data are of equal intensity and so the apportioning of the observed data amongst these contributing reflections is straightforward. The effect of DNA deuteration on the observed diffraction data is clear from Fig. 2 which shows a comparison between the data recorded in this study of deuterated A-DNA hydrated with D_2O (Fig. 2a) and that published by Langan et al. [17] for hydrogenated A-DNA hydrated with D_2O (Fig. 2b).

3.1. Imaging techniques

The imaging techniques used in this study closely follow well-established crystallographic methods whereby the observed data are used in conjunction with a set of structure factors calculated from an established model of part of the structure to produce Fourier synthesis and Fourier difference synthesis maps. In this work the initial structure factor amplitudes and phases were calculated from the coordinates of a refined model for the A-DNA double helix [27], using coherent neutron scattering lengths for the atoms making up the deuterated structure.

At the resolution of a typical neutron high-angle fibre diffraction study of hydrogenated DNA (~ 3 Å), the negative coherent scattering length of hydrogen tends to cancel with the positive scattering lengths of surrounding atoms so that, for example, sugar groups (which contain five carbon atoms, one oxygen atom, and seven hydrogen atoms) are weakly imaged in Fourier maps. Since in this study the

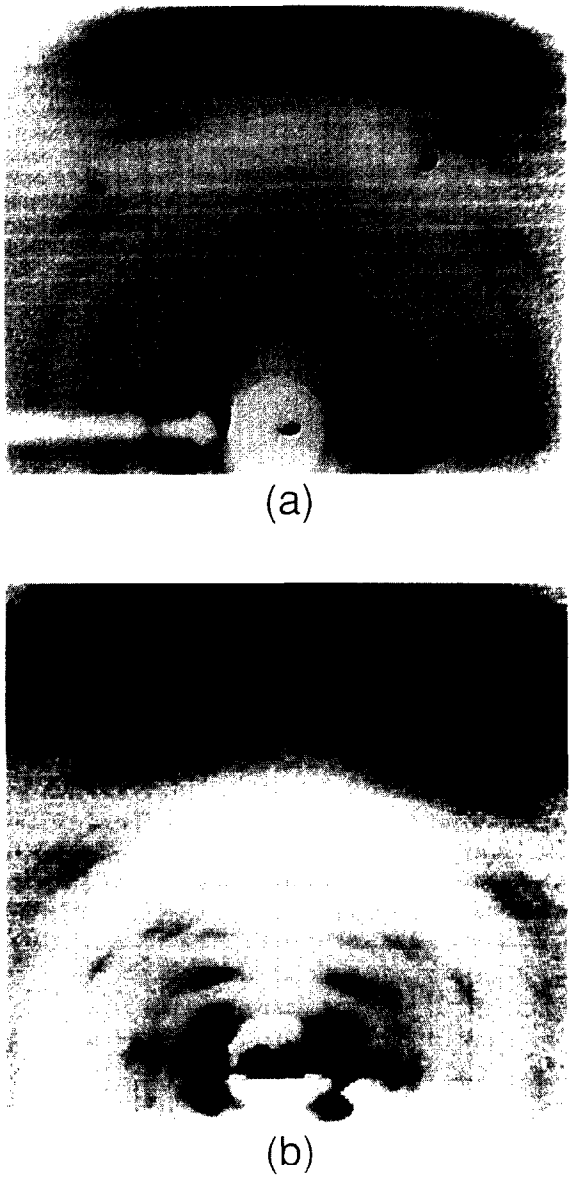

Fig. 2. Comparison of neutron high-angle fibre diffraction patterns recorded for (a) the hydrogenated A conformation hydrated with D_2O [17], and (b) the deuterated A conformation hydrated with D_2O. The recorded data are shown mapped into 'flat-film' geometry.

Fig. 3. 'Omit'-Fourier-difference map calculated using structure factors and phases derived from a model of deuterated A-DNA in which the sugar groups were removed. The sugar groups are clearly visible in this map, reflecting their contribution to the observed structure factor amplitudes.

hydrogen atoms covalently bound to the DNA were replaced by deuterium, it was expected that the contribution to the observed diffraction data due to the deuterium atoms of the sugar group would be significant. A model of deuterated A-DNA in which the atoms of the deoxyribose sugar had been removed was constructed and structure factor amplitudes and phases calculated from this model used in conjunction with the observed data to produce an 'omit map'. As can be seen in Fig. 3 the resulting map exhibited clear density peaks corresponding to sugar groups along the helix. These results demonstrate the validity of the imaging methods and also of the DNA model structure used to provide the initial set of phases.

3.2. *Imaging and refinement of ordered water sites*

The strategy adopted in the determination of the location of ordered water around the DNA was to use successive cycles of Fourier synthesis and Fourier difference synthesis followed by reciprocal space refinement of the positional and occupancy parameters of water peaks identified from these maps. The refinement procedure exploited the downhill simplex algorithm of Nelder and Mead [28], and minimised the R-factor,

$$R = \frac{\sum_{hkl} | KF_{obs} - F_{calc} |}{\sum_{hkl} KF_{obs}}$$

Fig. 4. Fourier difference synthesis map showing the location of site 1 water peaks which occur between O1 phosphate oxygen atoms of successive residues on each strand.

Fig. 5. Fourier difference synthesis map showing the location of site 2 water peaks in the centre of the major groove at a radius of 8.3 Å (measured from the helix axis).

Fig.4

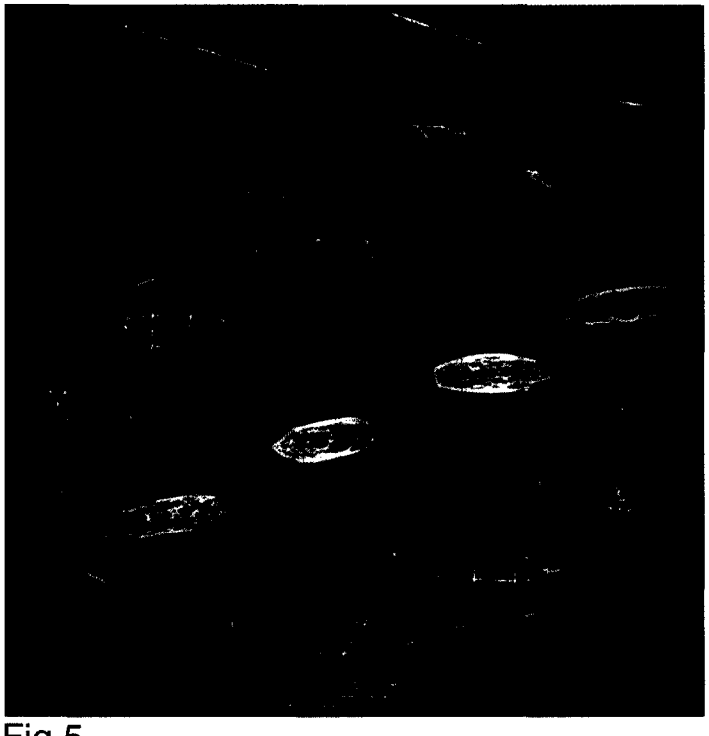

Fig.5

where F_{obs} and F_{calc} were the observed and calculated structure factor amplitudes respectively and K was a factor required to place the observed data on the same scale as the calculated. Water identified from Fourier difference maps was included in subsequent structure factor calculations by means of a spherically averaged scattering factor for D_2O.

The first Fourier synthesis and Fourier difference synthesis maps showed two particularly distinct sets of peaks in the major groove. Both sets exhibited the 11-fold helical symmetry characteristic of the A conformation of DNA. The first group of peaks (site 1), were located between phosphate O1 atoms of successive residues on the same strand and the edges of the bases in the major groove. The second set of peaks (site 2) appeared at the centre of the opening of the major groove. Views of the Fourier difference synthesis maps showing sites 1 and 2 can be seen in Figs. 4 and 5 respectively. The positional and occupancy parameters of both sites 1 and 2 were then refined while imposing 11-fold screw symmetry constraints.

A model of deuterated A-DNA incorporating ordered water sites 1 and 2 with their refined positions and occupancies was then constructed and the new set of structure factors used to calculate a further Fourier synthesis and Fourier difference synthesis. From the resulting density maps, two additional sets of peaks were identified. One of these (site 3) was located in the centre of the major groove at a smaller radius than site 2 and also exhibited the helical symmetry of the DNA. A fourth site was indicated by the presence of a continuous core of density running along the helix axis in the major groove. Views of the Fourier difference synthesis maps showing sites 3 and 4 can be seen in Figs. 6 and 7 respectively.

The positional and occupancy parameters of all of these sites were then refined together, with constraints imposed to maintain 11-fold screw symmetry about the helix axis. The refined positions and occupancies of the ordered water sites corresponding to the residue at $z = 0$ are given in Table 1. During this refinement it was found that, whereas the positions and occupancies of sites 1, 2, and 3 refined to the same values independent of starting point, the position of site 4 was able to move along the z-axis with a negligible change in R-factor. While not affecting the refined occupancy of site 4, its z coordinate could not be determined. This effect was consistent with the appearance of site 4 as a continuous column of density running along the helix axis of the DNA. The inclusion and refinement of ordered water sites 1–4 resulted in a significant reduction in the R-factor. The R-factor was reduced from 52.2% to 42.6% on the inclusion of unrefined sites 1 to 4 and finally to 36.5% after position and occupancy refinement of the ordered water sites. This R-factor is comparable to those obtained in X-ray fibre diffraction analyses of DNA at similar resolutions where water was included in the structure as a continuum [1,27].

In order to avoid 'overfitting' the observed diffraction data as the number of model parameters was increased, the observed reflections were separated into two unique sets; a 'test' set T and a 'working' set A. The test set T was obtained from a random selection of 10% of the observed reflections. As each of the ordered water sites were identified, in addition to refinement of positional and occupancy parameters against the full set of observed reflections, a parallel refinement was also performed against the working set of reflections A, and a 'free' R-factor (R_T^{free}) [29] was calculated from the test set T. Therefore R_T^{free} was independent of any overfitting of set A and the reduction of R_T^{free} at each stage of refinement provided an indication that the observed data had not been overfitted. The final positional and occupancy parameters of sites 1 to 4 calculated from refinement against the full set of observed reflections and against working set A were

Fig. 6. Fourier difference synthesis map showing the location of site 3 water peaks in the centre of the major groove at a radius of 4.5 Å (measured from the helix axis).

Fig. 7. Fourier difference synthesis map showing the site 4 'water column' which occurs as a continuous column of density along the helix axis.

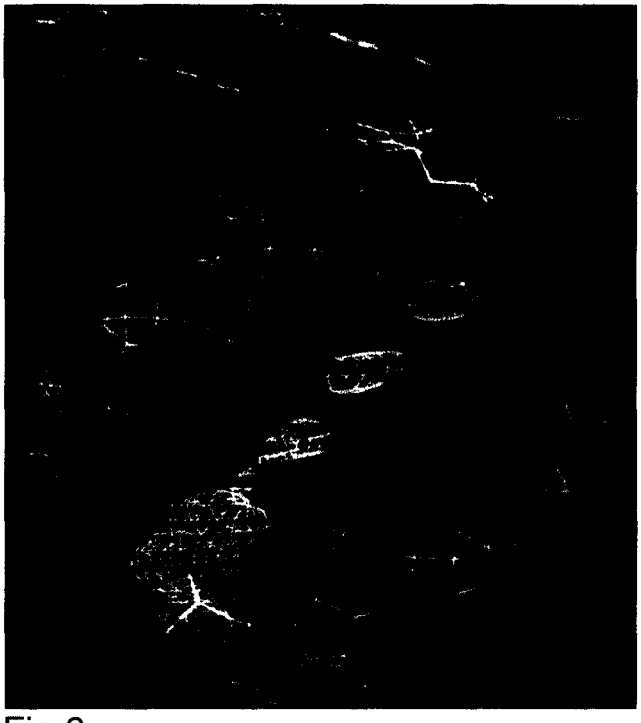

Fig.6

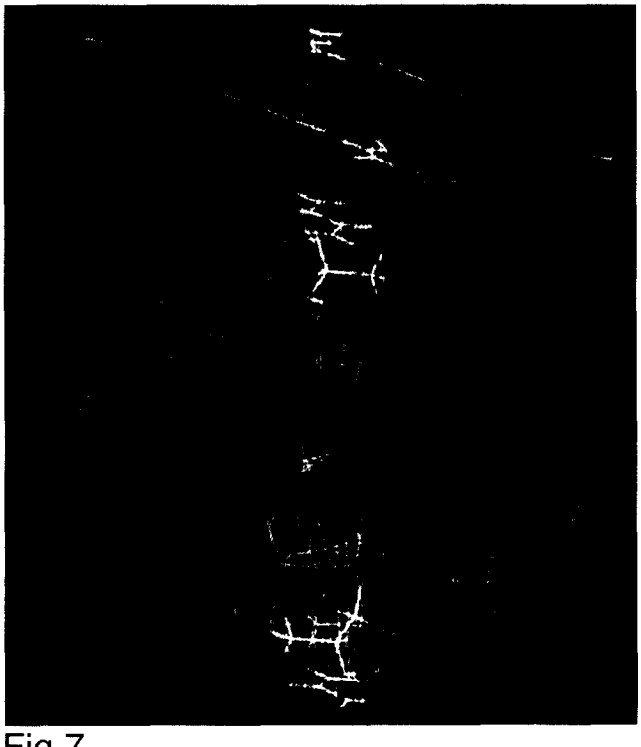

Fig.7

Table 1
The refined positional and occupancy parameters for water sites 1–4. The Cartesian axes were chosen such that the a-axis of the unit cell lies in the xz plane and the b-axis lies along the y-axis. Symmetry-related positions can be generated by using the 11-fold screw symmetry about the z-axis with the molecular dyad in the plane of the base-pair oriented along the y-axis

Site	x (Å)	y (Å)	z (Å)	Occupancy
1	−4.580	0.271	−2.696	1.32
2	4.577	−6.971	2.597	1.07
3	−1.300	−4.358	−1.300	0.82
4	0.000	0.000	–	0.77

identical. The various R-factors resulting from each stage of refinement are presented in Table 2. The significance of the reduction of the R-factor at each stage of model augmentation was also tested using the Hamilton significance test [30]. This test indicated that the probability of error in accepting the augmented models produced by the inclusion of ordered water sites 1 and 2, and then 3 and 4, was less than 5%.

In the final stages of analysis the 11-fold noncrystallographic helical symmetry constraints were removed, and the location of all of the water sites in the unit cell refined independently. While this refinement resulted in a further drop in the R-factor to 33.54%, there was very little deviation from helical symmetry with the mean positional deviation from the helically constrained refinement being less than 0.5 Å and the mean deviation in occupancy being 0.03. The Hamilton significance test [30] indicated that the helically constrained model could not be rejected even at the 50% confidence level (i.e., the probability of error in rejecting the constrained model was greater than 50%).

Table 2
As each of the ordered water sites were identified from Fourier synthesis and Fourier difference synthesis maps, their positional and occupancy parameters were refined against the full set of observed data, yielding the R-factor R. Also a 'test' set of reflections T was obtained from a random selection of 10% of the observed reflections and omitted from a parallel refinement of positional and occupancy parameters against the 'working' set of reflections A, which yielded the R-factor R_A. A 'free' R-factor [29] (R_T^{free}) was calculated for the T set of reflections and the reduction in R_T^{free} on the inclusion and refinement of ordered water sites 1 to 4 is taken as an indication that the observed data was not 'overfitted' as the number of model parameters was increased

Sites included in refinement	Increase in no. of parameters	R	R_A	R_T^{free}
None	–	52.2	52.2	51.5
1 and 2	8	43.9	43.9	41.4
1, 2, 3 and 4	8	36.5	36.6	37.2

4. Discussion

The four water sites identified in this study have been considered in terms of possible interactions with the DNA. It is important to emphasise that the DNA used in this study was effectively of 'random sequence' so that any sequence-dependent features were averaged in the diffraction data and also in the Fourier maps generated from these data. Such sequence averaging must be considered carefully when suggesting possible water–DNA hydrogen bonding interactions that are consistent with the experimental observations and with the available hydrogen-bonding donor and acceptor sites on the DNA. All water–DNA distances within the range of 2.2 Å to 3.8 Å have been considered to be within the range of hydrogen bonding in order to allow for some error in the refined positions of the sites.

Site 1 is located in the major groove, at equal distances from neighbouring phosphate O1 atoms on the same strand. This site was a prominent feature in the work described by Langan et al. [17], and is consistent with the intrastrand water bridging of phosphate O1 atoms in A-DNA. The presence of such water bridges has been frequently observed in X-ray diffraction studies of oligonucleotide single crystals [10,11]. Site 1 was also located within possible hydrogen bonding distance of the purine N7 atom in the major groove and with an occupancy of 1.32 it is possible that this feature may reflect the involvement of an additional water molecule interacting either between water bridges linking successive O1 atoms and the purine N7 or between the O1 oxygen itself and the purine N7. Since the observed data is sequence averaged, this additional interaction would be expected to occur with a weight of 0.5. Water bridges between phosphate O1 and purine N7 atoms have been previously observed in single-crystal

studies [31,32]. It should be noted that the thymine methyl carbon atom is located at a distance of 2.2 Å from the site 1 position. The proximity of the methyl group may cause local disturbances in the order of site 1 water molecules which cannot be identified from this sequence averaged data set. There is also the possibility of stabilising hydrophobic interactions between the methyl group and water molecules at this site. Such interactions have been previously observed in single-crystal studies of B-DNA [33]. Site 2 was located at the centre of the opening of the major groove at equal distances from phosphates on either strand. However the O1–O1 and O2–O2 distances between opposite strands of 6.65 Å and 7.06 Å, respectively appear to be rather wide to be bridged by a single water molecule engaged in bidentate hydrogen bonding, and it is possible that other interactions involving the cations around the DNA may be involved in stabilising this site. In an X-ray fibre diffraction study of the positions of thallium cations around A-DNA [34], cations were observed to zig-zag across the opening of the major groove between charged phosphates on opposite strands. It is noteworthy that a network of solvent molecules stretching across the opening of the major groove was previously observed in an X-ray diffraction study of the oligonucleotide d(CpCpGpG) [14].

Site 3 positions were found in the centre of the major groove, at a smaller radius than site 2. These peaks were not located within 3.8 of any DNA atoms. However the site 3–site 1 and site 3–site 3 distances were comparable to those associated with typical water–water hydrogen bonding [35]. Site 3 water may also be associated with a string of cations which in the X-ray study of thallium A-DNA were found to lie along the centre of the major groove [34].

Site 4 appeared as a continuous core of density running along the helix axis in front of the bases in the major groove, as observed in the previous neutron fibre diffraction study of hydrogenated A-DNA [17] and in a single crystal study of the RNA A-type double helix $[U(UA)_6A]_2$ [32]. This site was located within possible hydrogen bonding distance of adenine NH_2, thymine O4, cytosine NH_2 and guanine O6 and may therefore be involved in hydrogen bonding interactions with any of these hydrophilic groups. However a complete interpretation of this feature was not possible as a result of the sequence averaging of the base pairs.

In this study we were unable to verify two of the weaker water features identified in the previous neutron high angle fibre diffraction study of hydrogenated A-DNA [17] where they were identified as features 2 and 3. This difference in the results from the two studies can be attributed to the improved diffraction data recorded in the work described here where the use of deuterated DNA has resulted in the reduction of incoherent scattering and absorption effects.

Two of the hydration features identified in this study may involve sequence-specific interactions with DNA base atoms. Site 1, although sequence independent in terms of the bridging of successive O1 phosphate oxygens, occupies a position where purine N7 atoms and thymine methyl groups may be important to its stability. Similarly, the site 4 water 'column' occurs in a position where there are numerous possible interactions with the exposed hydrophilic groups of the bases in the major groove. The sequence averaging inherent in this analysis means that it is not possible to determine these sequence-specific features from the current work. However this information should be available from neutron high-angle fibre diffraction studies of the A conformation of DNA containing regular repetitive base-pair sequences. Further investigations of A-DNA hydration will therefore focus on elucidating this sequence dependent variation in studies of synthetic DNA polynucleotides such as poly[d(A-T)] · poly[d(A-T)], poly[d(G-C)] · poly[d(G-C)], and poly d(G) · poly d(C). Such studies will also be of interest in providing an understanding of the relative stabilities of the A conformation of these polymers. For example, whereas the A conformation is exceptionally stable in poly d(G) · poly d(C), it has not so far been reported at all for poly d(A) · poly d(T).

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