THE ROYAL SOCIETY

Published online 16 July 2004

Water–DNA interactions as studied by X-ray and neutron fibre diffraction

Watson Fuller[1*], Trevor Forsyth[1,2] and Arumugam Mahendrasingam[1]

[1]*School of Chemistry and Physics, Keele University, Keele ST5 5BG, UK*
[2]*Institut Laue-Langevin, 6 Rue Jules Horowitz, 38042 Grenoble Cedex 9, France*

X-ray fibre-diffraction studies indicate a high degree of stereochemical specificity in interactions between water and the DNA double helix. Evidence for this comes from data that show that the molecular conformations assumed by DNA in fibres are highly reproducible and that the hydration-driven transitions between these conformations are fully reversible. These conformational transitions are induced by varying the relative humidity of the fibre environment and hence its water content. Further evidence for stereochemical specificity comes from the observed dependence of the conformation assumed on the ionic content of the fibre and the nucleotide sequence of the DNA. For some transitions, information on stereochemical pathways has come from real-time X-ray fibre diffraction using synchrotron radiation; information on the location of water with respect to the double helix for a number of DNA conformations has come from neutron fibre diffraction. This structural information from fibre-diffraction studies of DNA is complemented by information from X-ray single-crystal studies of oligonucleotides. If the biochemical processes involving DNA have evolved to exploit the structural features observed in DNA fibres and oligonucleotide single crystals, the challenges in developing alternatives to a water environment can be expected to be very severe.

Keywords: DNA; water; X-ray fibre diffraction; neutron fibre diffraction

1. INTRODUCTION

This paper focuses on the information obtained from X-ray and neutron fibre diffraction on the interactions of water and ions with DNA. Particular emphasis is placed on the high degree of reproducibility in the changes observed in the conformation and organization of molecules of DNA in fibres, in response to changes in the degree of hydration. This reproducibility implies a high degree of specificity in the interaction of water with DNA and provides a powerful stimulus for characterizing, in as much detail as possible, the changes in DNA conformation induced by changes in hydration and the location of water with respect to the DNA in its various conformations. In the context of this discussion it is important to recognize that the resolution of fibre-diffraction data from DNA is rarely better than 2 Å, i.e. comparable to the length of the hydrogen bonds through which water molecules interact with each other and with hydrogen bond donor and acceptor groups in macromolecules such as DNA. Despite this limitation, fibre diffraction has made crucial contributions to characterizing the various conformations assumed by DNA molecules and identifying the factors that are important in stabilizing conformations and inducing transitions between them. Although fibres do not have the full three-dimensional order in their molecular arrangement characteristic of single crystals, they do offer significant advantages for studying transitions between conformations and changes in molecular arrangement. The fibrous state, with its typical mix of highly ordered and amorphous regions, can accommodate changes in molecular conformation and packing much more readily than can single crystals. This capability allows a systematic investigation of the dependence of molecular conformation on the water and ionic contents of a fibre. In particular, conditions can be chosen that are much closer to the *in vivo* environment than is typically achieved in solution or in single-crystal studies. Since changes in the water content of a fibre can be controlled by varying the relative humidity of the fibre environment, studies of fibres offer the possibility of time-resolved studies of stereochemical pathways followed in structural transitions. The exploitation of the high brightness of X-ray synchrotron radiation sources in which a diffraction pattern can be recorded in seconds or minutes, rather than the hours or days required when using a conventional source, is described in § 3. The ability to vary the water content of the fibre by controlling the relative humidity of its environment has also been exploited in neutron fibre-diffraction studies, in which the H_2O in the fibre is replaced with D_2O and which, through the use of Fourier difference syntheses, have allowed the location of water molecules within ordered regions of DNA fibres to be determined.

2. HISTORICAL PERSPECTIVE

X-ray fibre-diffraction studies of DNA by Wilkins and Gosling (referred to in Watson 1961) showed that the DNA molecule could assume a highly regular structure.

* Author for correspondence (w.fuller@keele.ac.uk).

One contribution of 16 to a Discussion Meeting Issue 'The molecular basis of life: is life possible without water?'.

However, for this to occur the fibre had to be kept moist. Although exposing fibres to a relative humidity of less than *ca*. 30% results in the loss of ordered structure, this loss is reversed when a fibre is re-wetted. Franklin & Gosling (1953*a*) developed techniques for controlling the relative humidity of the fibre environment and hence the water content of the fibre. With this facility they obtained better-defined diffraction patterns of the two types of pattern previously observed for DNA, which they designated A and B (figure 1). More importantly, they observed the pattern from a single fibre change from A to B when the relative humidity was increased from 75% to 92%. Some patterns were observed that were a mixture of A and B types. The occurrence of such mixtures was attributed to the fibre containing some regions with the DNA in the conformation that gave the A form and others with the DNA in the conformation that gave the B form. The two-stranded model proposed by Watson & Crick (1953) with its 10 nucleotide pairs per helix pitch of 34 Å accounted, in general terms, for the B-type pattern. More detailed analysis by Wilkins *et al.* (1953) and by Franklin & Gosling (1953*b*) showed that both the A and B forms could be accounted for by models of the Watson and Crick type but with the A form differing from the B form in having 11 rather than 10 nucleotide pairs per helix pitch and a pitch length of 28 Å rather than 34 Å. However, both structures had the negatively charged phosphate groups on the outside of the molecule.

Although the specific base pairing holding the two DNA chains together results in key functional groups being buried in the centre of the DNA double helix, some groups attached to edges of the bases not involved in base pairing are exposed to ions and water in the grooves of the double helix. X-ray fibre-diffraction studies of synthetic two-stranded DNAs with specific nucleotide sequences have shown that the dependence of double-helical structure on the degree of hydration and ionic environment is also influenced by nucleotide sequence with, in the case of the D (Arnott *et al.* 1983) and Z (Arnott *et al.* 1980) forms, new double-helical conformations being assumed (figures 1 and 2).

Of significance, from the point of view of characterizing structural transitions within the DNA double helix, is the fact that whereas the A form is fully crystalline (i.e. there is three-dimensional order within the crystalline regions of the fibre), the B form is semi-crystalline with screw disorder, in which neighbouring molecules within a hexagonally packed two-dimensional array are screwed in or out of the plane of the array by random amounts. The early X-ray fibre-diffraction studies were made on the sodium salt of DNA. In subsequent work by Wilkins and co-workers a great deal of effort was directed at studying the effect on the structures observed for the DNA double helix of the ionic content of the fibre, and in particular the nature of the alkali metal counterion that neutralized the phosphate groups. These studies resulted in much better defined X-ray fibre-diffraction patterns, which allowed models of both the A and B forms to be refined so that the position of the strongly scattering phosphate groups could be determined to an accuracy of a few tenths of an ångström (Langridge *et al* 1960*a*,*b*; Fuller *et al.* 1965). The recognition of the key roles played by ions, together with water, in determining the structure assumed by the DNA double helix emphasized the importance of treating DNA fibres as three-component systems, with ions and water interacting strongly with each other as well as with the DNA. Furthermore, it also became clear that it was not simply the alkali metal cations that played key roles in determining DNA structure. Halogen ions associated with excess cations, beyond those required for neutralization of phosphate groups, were also found to play crucial roles in determining DNA structure, for example, in the C form (Marvin *et al.* 1961).

The principal conformations of the DNA double-helical conformation are illustrated in figure 1, together with the X-ray fibre-diffraction patterns on which they were based. The dependence of double-helical conformation on nucleotide sequence is summarized in figure 2.

3. TIME-RESOLVED X-RAY FIBRE-DIFFRACTION STUDIES OF STRUCTURAL TRANSITIONS IN DNA DOUBLE HELICES

The increasing availability of X-ray synchrotron sources from the early 1980s dramatically extended the power of fibre-diffraction techniques. In particular, because such sources were typically many orders of magnitude more brilliant than the most intense laboratory sources, it became possible to record a fibre-diffraction pattern from a 100 μm diameter fibre of DNA in minutes rather than hours or days. These facilities made feasible time-resolved studies with the possibility of mapping the stereochemical pathways followed in structural transitions. The Keele Fibre Diffraction Group used the Daresbury SRS to follow transitions between the principal conformations of the DNA double helix. From the point of view of a discussion aimed at characterizing diversity in the ways in which water interacts with biological macromolecules, it is important to emphasize that there was considerable variety in the pathways followed. At one extreme the A ↔ B transition appeared to be similar to a first-order phase transition, with little evidence of intermediate structures; the time-resolved datasets recorded at the Daresbury SRS for this transition were of the A or B type or simple mixtures of the A and B patterns. By contrast, the D ↔ B transition exhibited a gradual distortion of the double-stranded helix as its pitch increased over a period of minutes from 24 Å to 34 Å (Forsyth *et al.* 1986; Mahendrasingam *et al.* 1986). However, the A ↔ B and D ↔ B transitions have a number of important features in common. In particular, if the ionic content is appropriate they can be induced by simply changing the relative humidity of the fibre environment and hence its water content, and they are both fully reversible.

These features are well illustrated by the data on the D ↔ B transition observed for the synthetic polynucleotide poly[d(A–T)].poly[d(A–T)], in figures 3 and 4 (Mahendrasingam *et al.* 1986). Figure 3 illustrates a selection from the series of patterns recorded during a D ↔ B transition. Pattern (*a*) is a highly crystalline D-type pattern in which the reflection marked I is related to the lateral intermolecular separation and that marked II to the helical pitch of the molecules in the D conformation. Patterns (*b–d*) contain reflections corresponding to an intermediate semi-crystalline form. In (*c*), the reflection marked III is related to the lateral intermolecular separation and that

marked IV to the helical pitch of molecules in the semi-crystalline intermediate form. Pattern (*f*) is a well-defined semi-crystalline B pattern with no traces of the semi-crystalline intermediate form. Measurements on the reflections identified in figure 3 for the series of patterns recorded during four complete D ↔ B ↔ D cycles are shown in figure 4. Figure 4 demonstrates the high degree of structural reversibility and reproducibility observed throughout this series of cycles and hence the high degree of specificity in solvent–DNA interactions. Each stage in the humidity cycle in figure 4 is characterized by a well-defined layer line spacing and hence helix pitch for the semi-crystalline intermediate form. The coexistence of crystalline D and semi-crystalline intermediate regions within the fibre can be related to the rate at which water penetrates the fibre. On this model, the regions where water has still not penetrated remain in the D form but in those regions where water has already penetrated all the molecules are, to a first approximation, in the intermediate semi-crystalline form with a pitch length characteristic of the current stage in the humidity cycle.

Figure 5 illustrates data on the D ↔ B transition in poly[d(A–T)].poly[d(A–T)] recently recorded at the Daresbury SRS using an online image plate detector (H. Ye and V. T. Forsyth, unpublished data). The numerous advantages of online detector systems in studies such as these is well illustrated by comparison of these data with those from the original studies of the D ↔ B transition illustrated in figure 3, which used photographic film recording. Pattern (*a*) in figure 5 is a highly crystalline D-type pattern. Patterns (*b–g*) show diffraction corresponding to intermediate structures recorded as the structure changes into the semi-crystalline B form. Pattern (*h*) is a well-defined semi-crystalline B pattern with no traces of the semi-crystalline intermediate structure. The improved time resolution and greater reproducibility in the detector performance has allowed more detailed analysis of this transition, based on the separation of crystalline and continuous diffraction components recorded during this transition using CCP13 software (Squire *et al.* 2003) and subsequent modelling work (H. Ye and V. T. Forsyth, unpublished data).

The observation that the two-stranded synthetic polynucleotide poly[d(G–C)].poly[d(G–C)] could assume a left-handed double-helical conformation (Wang *et al.* 1979; Arnott *et al.* 1980) inevitably raised questions about the stereochemical pathways that could be followed if transitions from this conformation (designated Z) to the right-handed B conformation were to occur. Fibre-diffraction experiments to address this were performed by Mahendrasingam *et al.* (1990), who showed that for poly[d(G–C)].poly[d(G–C)] in a high-salt environment transitions between the left-handed Z form and the right-handed B form could indeed be observed as a function of relative humidity of the fibre environment, and furthermore that these transitions were reversible.

Further interest in transitions that involve a change of sense of the DNA double helix has been generated by the observation that the polynucleotide poly[d(G–m^{5}C)].poly[d(G–m^{5}C)] can undergo a transition between the A and the Z conformations at much lower conditions of excess salt than that required for the Z ↔ B transition in the non-methylated analogue (V. T. Forsyth, unpublished data). Of further interest was the fact that this transition, as observed in fibres, appeared to be *irreversible*. Figure 6 shows a sequence of diffraction patterns recorded during the A → Z transition. As for the D ↔ B transition, the A → Z transition also shows evidence of continuous changes in helix geometry starting with a crystalline A form in figure 6*a*, moving into a semi-crystalline Z form at high humidity (figure 6*f*). As the humidity is reduced the DNA remains in the Z conformation, crystallizing into a hexagonal lattice at lower humidities (figure 6*i*). These observations, together with the fact that methylation of alternating G–C sequences is believed to be important in regulatory processes, suggest an important role for such structural transitions in biological processes *in vivo*.

It is difficult to envisage a complete polymer chain undergoing a transition from a left-handed to a right-handed helical conformation within a fibrous structure and it may be that reversal of handedness is limited to relatively short segments of the polymer associated with changes in the distribution of the polymer between more-ordered and less-ordered regions of the fibre. However, the crucial point for this discussion of specificity in water–DNA interactions is that humidity-driven changes in the handedness of the DNA double helix in fibres are highly reproducible for comparable rates of change in the humidity of the fibre environment, once again highlighting the high degree of specificity in the role of water in inducing conformational transitions in DNA.

The limiting factors in time-resolved studies of structural transitions in DNA double helices like those described above are the X-ray flux at the specimen and the cycle time of the detector used to record the diffraction data. Electronic area detectors have advantages over film, not only in speed but also in their capacity to display data essentially as it is recorded in real time. The gains in these two factors have a further important consequence in that they allow data to be recorded in acceptable time-scales with fibres of much smaller diameter for which the degree of hydration of the DNA can respond much more rapidly to changes in the relative humidity of the environment of the fibre. In addition to allowing diffraction from thin fibres to be recorded in acceptable times, beam line ID02 at the European Synchrotron Radiation Source (ESRF) also allows both low- and high-angle data to be recorded simultaneously so that changes in molecular conformation, as indicated by the diffraction data in figures 3, 5 and 6, can be correlated with changes in molecular organization indicated by low-angle data. Recent experiments on the transition between the A and B conformations of *Escherichia coli* chromosomal DNA, taking advantage of the much improved time resolution of ID02, suggest that a continuous pathway of conformational intermediates occurs over a period of a few seconds, well beyond the time resolution available in previous work on this transition (I. M. Parrott, V. Urban and V. T. Forsyth, unpublished data).

4. LOCATION OF WATER MOLECULES FROM NEUTRON FIBRE DIFFRACTION

In the determination of the structure of the DNA A and B conformations, the water surrounding the DNA was treated as a continuum (Langridge *et al.* 1960*a*,*b*; Fuller *et al.* 1965). However, in modifying the X-ray atomic-

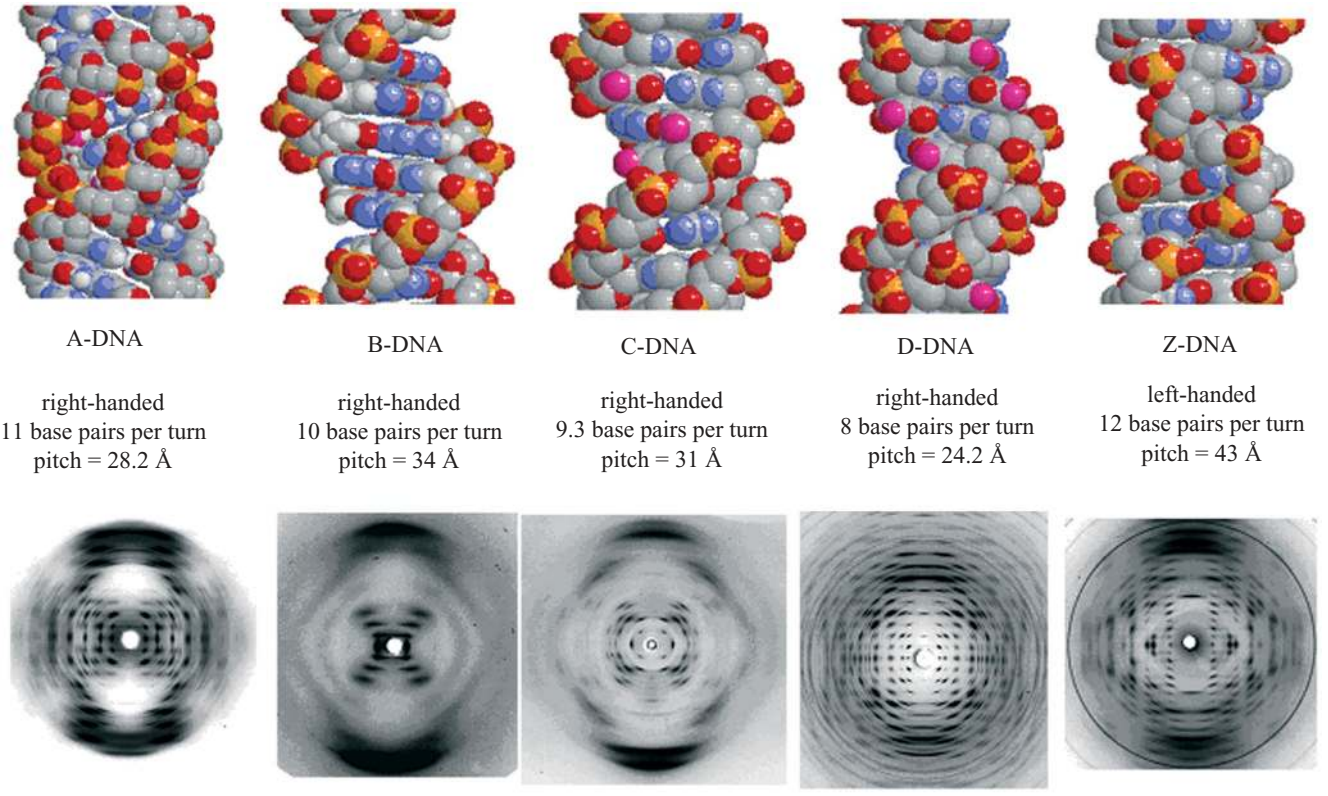

Figure 1. The five major conformational variants of the DNA double helix, their helix parameters and their X-ray fibre-diffraction patterns. The A, B, C, D and Z structures are described in Fuller *et al.* (1965), Langridge *et al.* (1960 *a* and *b*), Marvin *et al.* (1961), Arnott *et al.* (1983) and Arnott *et al.* (1980), respectively.

natural DNA	alternating A–T	alternating G–C	alternating G–m^{5}C	homopolymer G–C	homopolymer A–T
random sequence	A–T T–A A–T T–A A–T T–A A–T T–A A–T T–A	G–C C–G G–C C–G G–C C–G G–C C–G G–C C–G	G–m^{5}C m^{5}C–G G–m^{5}C m^{5}C–G G–m^{5}C m^{5}C–G G–m^{5}C m^{5}C–G G–m^{5}C m^{5}C–G	G–C G–C G–C G–C G–C G–C G–C G–C G–C G–C	A–T A–T A–T A–T A–T A–T A–T A–T A–T A–T
C ⟷ A ⟷ B	D ⟷ B	Z ⟷ B	A ⟶ Z	A	B

Figure 2. Diagram summarizing the structures adopted in simple polymeric DNA sequences, as observed by fibre diffraction. The middle section shows the repeat in the polymer; the bottom section shows the structures most commonly adopted, together with indications of the most commonly occurring transitions that occur as a function of the fibre environment.

scattering factors to allow for this, it was recognized that the closeness with which water molecules could be expected to approach particular groups in the DNA would be influenced by the hydrophobicity of the group. This resulted in the effective volume of the strongly hydrophilic phosphate groups being reduced, with a corresponding increase in their effective electron density and hence X-ray scattering power. By contrast, hydrophobic groups such as the sugar rings would have an increased effective volume and a corresponding reduction in their effective electron density and hence in their scattering power. Marvin (1960), Arnott *et al.* (1965) and Marvin *et al.* (1966) pioneered the use of Fourier synthesis techniques in the analysis of fibre-diffraction data from the crystalline B form of lithium DNA. Although features in difference maps close to the DNA, which might have been identified with water

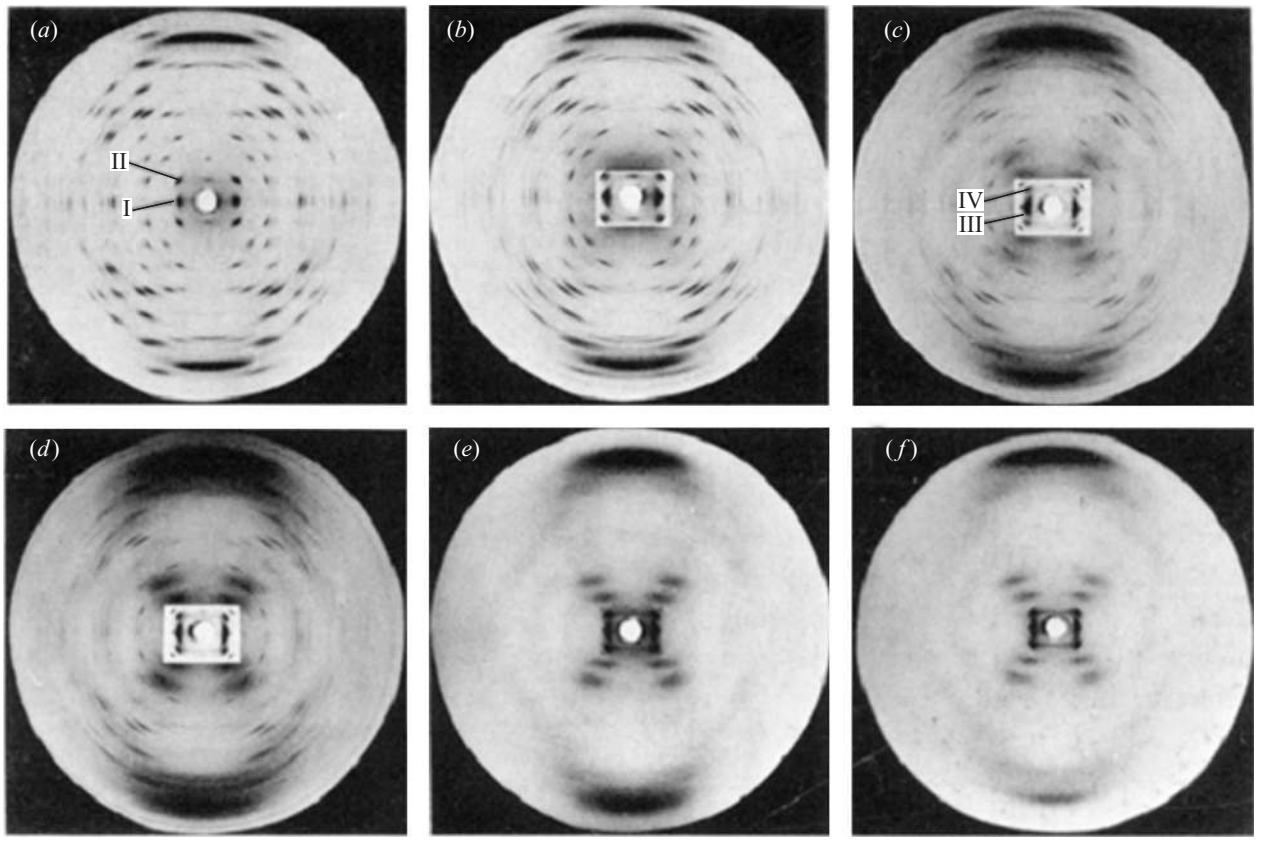

Figure 3. Diffraction pattern illustrating typical stages in the D ↔ B transition (see § 3 for details of the reflections identified as I, II, III and IV in these patterns). In the reproduction of patterns (*b*), (*c*) and (*d*) diffraction in the central region of the pattern has been attenuated so that neighbouring reflections are more easily resolved.

molecules and ions in relatively fixed positions, were unconvincing, these techniques proved to be extremely successful in refining features in the DNA double helix.

The increasing availability of intense neutron beams from the late 1970s offered the possibility of employing Fourier difference techniques to locate water around DNA by exploiting the relatively high scattering power of neutrons by hydrogen, and even more significantly by exploiting isotopic replacement of hydrogen by deuterium. The Keele Fibre Diffraction Group in experiments at the Institut Laue Langevin used established techniques for controlling the relative humidity of the fibre environment and hence the degree of hydration of the DNA, to replace the water surrounding the DNA by D_2O. The first experiments of this type were carried out on fibres of D-DNA (Forsyth *et al.* 1989; Fuller *et al.* 1989). These experiments clearly demonstrated that this isotopic replacement of light by heavy water caused significant, reversible and reproducible changes in the observed neutron fibre-diffraction patterns that could be used to image the location of ordered water by difference Fourier and Fourier synthesis methods. Furthermore, the fact that these changes extended throughout the resolution range of the observed diffraction patterns indicated that the water in the DNA polymer structures occupied well-defined positions. Figure 7 illustrates the change in the diffraction pattern recorded from an array of fibres of poly[d(A–T)].poly[d(A–T)] in the D form when water surrounding the DNA is replaced by D_2O.

Because neutron beams have a lower brilliance than X-ray synchrotron beams the data in figure 6 were recorded from a specimen consisting of some 40–100 μm diameter fibres mounted in a parallel array. Despite the more limited definition in the neutron-diffraction patterns, the basic similarity between them and the X-ray pattern in figure 5*a* is clear, as are the marked changes in the intensities of reflections in the neutron patterns when H_2O is replaced by D_2O. These changes were highly reproducible and, when the D_2O was replaced by H_2O, fully reversible. The difference map calculated using the data in figure 7 is illustrated in figure 8. The image of localized water in figure 8 is convincing because the features in it are not found in regions of the unit cell occupied by DNA but are nevertheless close to DNA groups in positions typical of a bound shell. However, the resolution of the data used in these experiments is such that in general it would not be justified to identify peaks in these maps with individual rather than groups of water molecules.

Figure 9*a* shows a difference Fourier map illustrating the location of water in the minor groove of the D conformation but with the threshold set so high that only the peaks in the density distribution are displayed. Figure 9*b* shows the same water features together with cation positions determined by X-ray isomorphous-replacement fibre diffraction (Forsyth *et al.* 1990). The overall picture for hydration in the minor groove of D-DNA is of an alternating string of water and cations along the length of the groove, interacting with each other and with base edge atoms.

Similar studies to those described for the D conformation of DNA have been undertaken on the A and B conformations (Langan *et al.* 1992; Shotton *et al.* 1997;

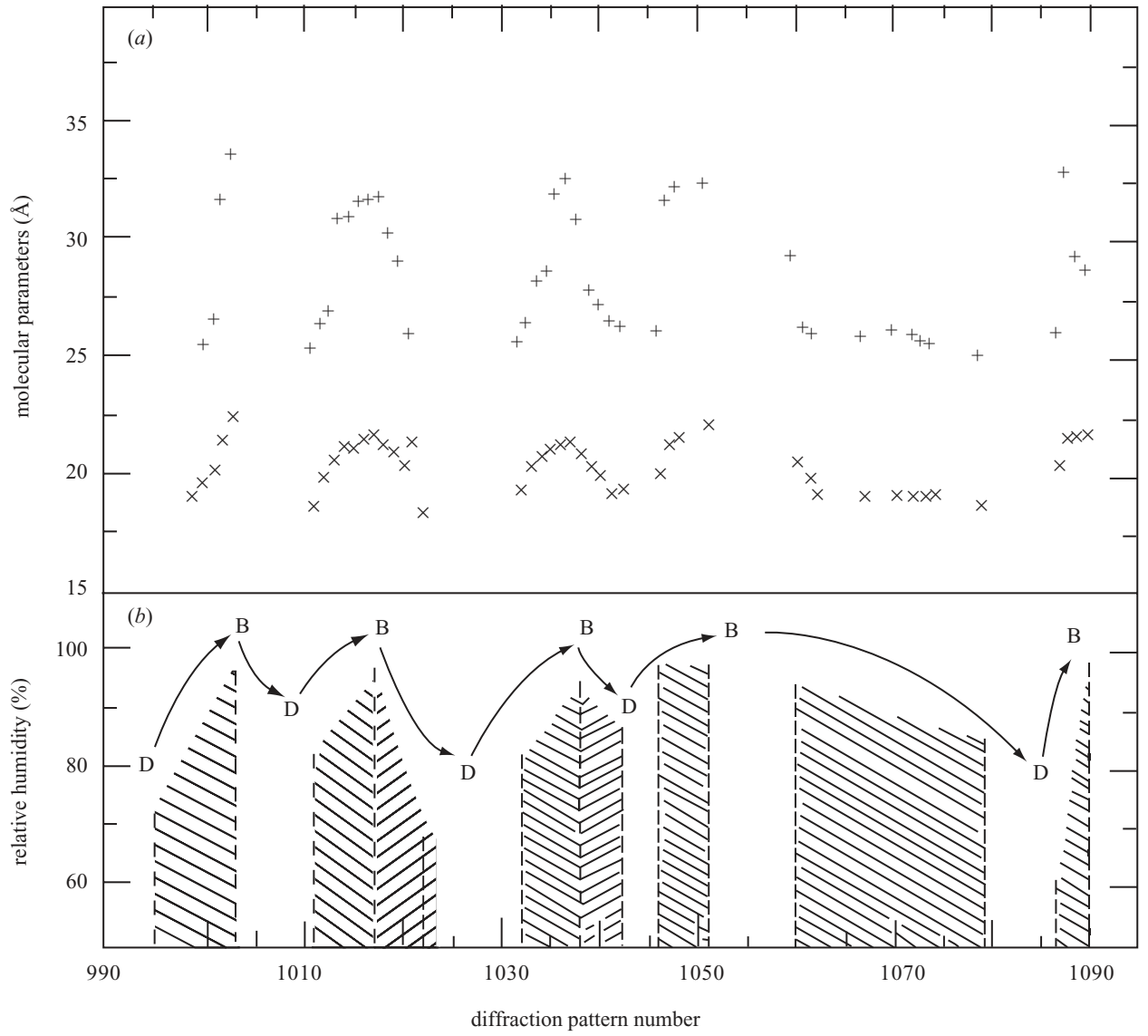

Figure 4. Variation in the lateral intermolecular spacing (crosses) and helix pitch (plus signs) of the intermediate crystalline form as a function of relative humidity. Approximately 100 diffraction patterns were recorded with exposures of 4–10 min, depending on the intensity from the SRS, over a period of 17 h during which four reversible transitions between the crystalline D and the semi-crystalline B forms were observed. In the gaps between the shaded regions, the conformation was maintained constant in either the B or D form.

Pope *et al.* 1998; Forsyth *et al.* 1998). Initial work on the hydration of the A conformation used hydrogenated material. The Fourier maps showed a number of features observed in X-ray diffraction studies of oligonucleotide single crystals. One of the most prominent features was the presence of water molecules between successive phosphate groups along the major groove. This hydration feature is shown clearly in the $(2F_o - F_c)$ Fourier map in figure 10.

The more recent study by Shotton *et al.* (1998*a*) showed the major advantages of using perdeuterated DNA for work of this type. Here, the DNA was obtained from *E. coli* cells that had been grown in D_2O, with a deuterated carbon source. Deuteration has very substantial advantages in neutron-scattering work (Forsyth *et al.* 2001) either as a label or, as in this case, as a way of eliminating hydrogen incoherent scattering, which appears as a 'background' in the diffraction patterns and limits the accuracy with which the Bragg reflections can be determined.

The summary in figure 11 of the main results obtained by Shotton *et al.* (1998*a*) identifies the following four main families of peaks in the water distribution:

(i) a site located in the major groove at equal distances from successive phosphate groups along the same strand;
(ii) a site located at the opening of the major groove at equal distances from phosphates on either strand;
(iii) a site also located at the centre of the major groove, but at a smaller radius;
(iv) a feature running down the 'hollow' centre of the molecule within possible hydrogen bonding distance to base edge atoms. This feature could not be completely interpreted as a result of the sequence averaging of the base pairs.

The column of water running down the centre of the DNA structure is of particular interest. The A confor-

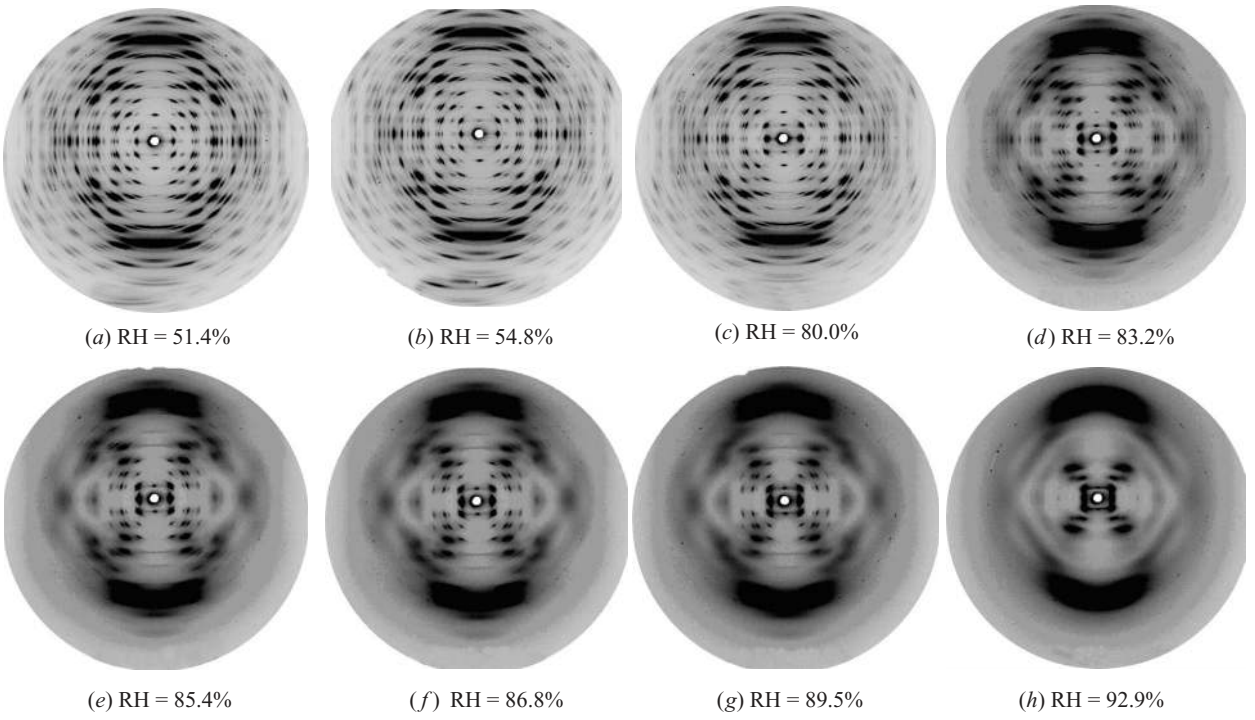

Figure 5. X-ray fibre-diffraction patterns recorded during a water-driven transition between the D and the B conformations of DNA. The data were recorded at the Daresbury SRS using an online image plate system (see text for description of the features in the diffraction patterns).

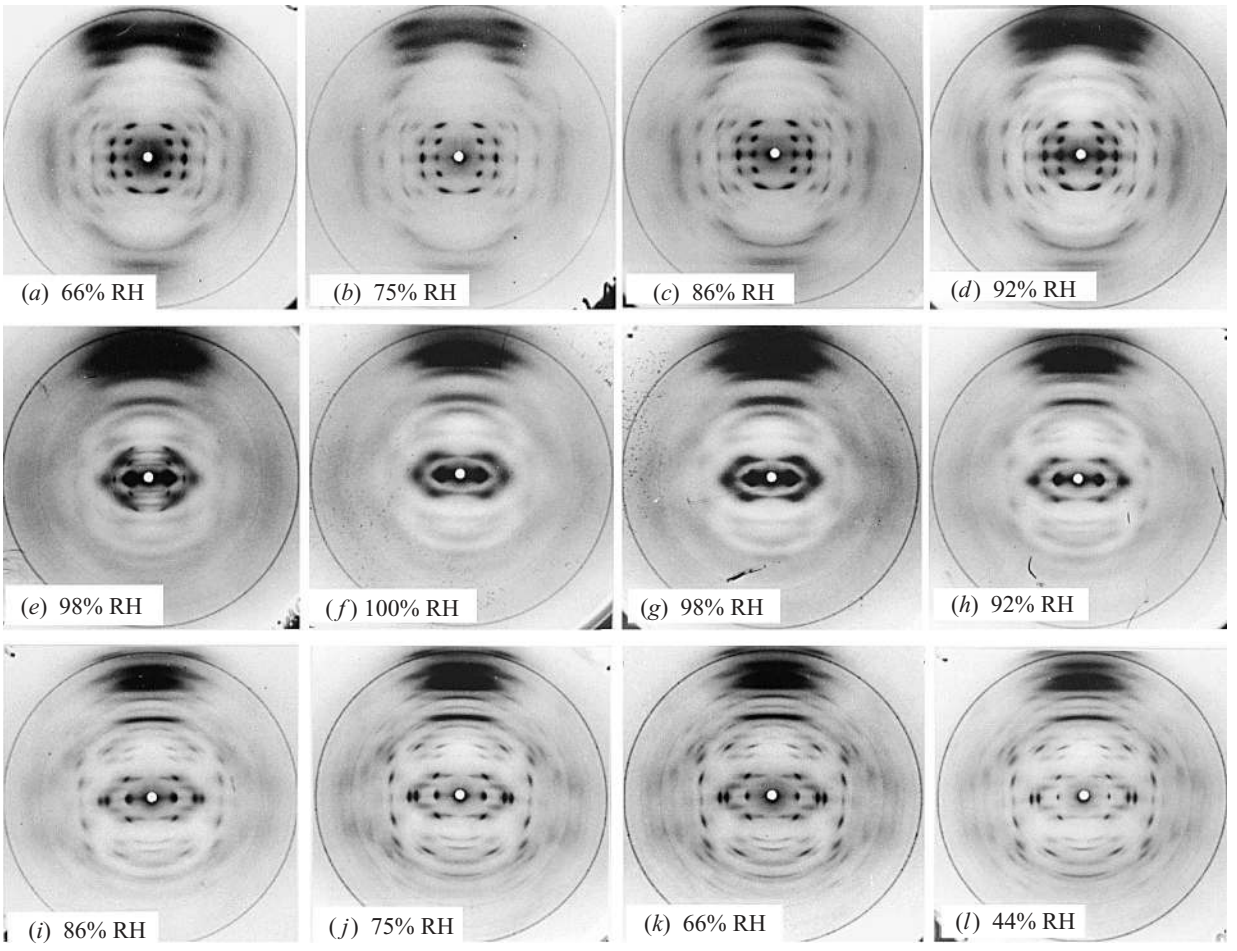

Figure 6. Series of diffraction patterns recorded during the water-driven transition between the A and Z conformations of poly[d(G–m^{5}C)].poly[d(G–m^{5}C)] (see § 3 for discussion of the features in the diffraction patterns). RH, relative humidity.

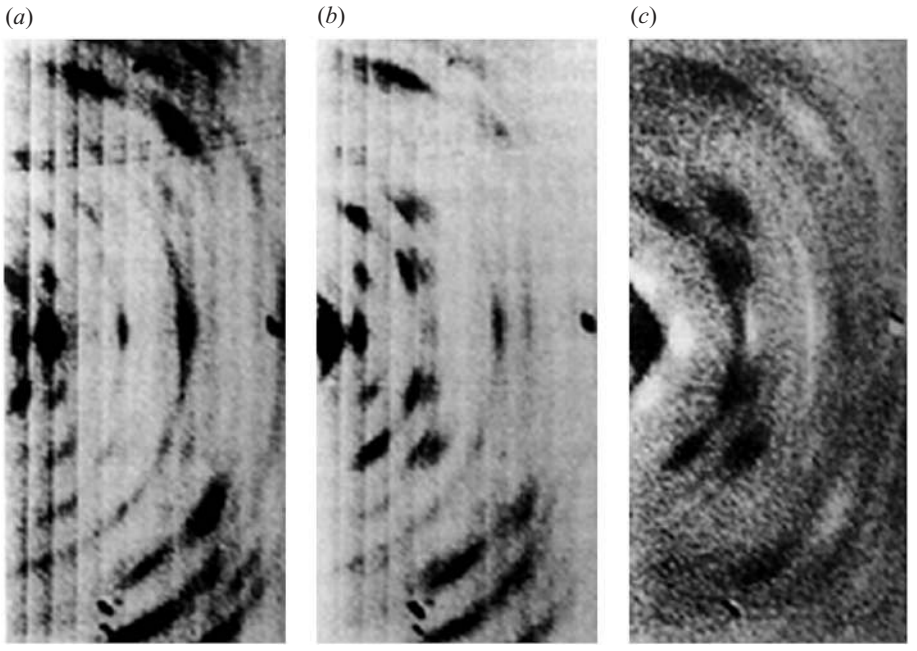

Figure 7. Neutron fibre-diffraction patterns from the D form of poly[d(A–T)].poly[d(A–T)]. (*a*) DNA surrounded by H_2O; (*b*) DNA surrounded by D_2O; and (*c*) point-by-point difference between the H_2O and D_2O patterns.

mation is described as having a 'hollow centre' because the base pairs in this conformation are *ca.* 5 Å from the helix axis so that a projection of the macromolecule down the helix axis has a marked central hole and the projection of the central column of localized water in figure 11*d* lies within this 'hole'. The apparent continuity within the central column of water and its close association with DNA suggests that a considerable degree of cooperativity may be associated with the formation and disruption of this column. Further, since in marked contrast to A, the base pairs in the B conformation lie on the helix axis, the characteristic central column of water could not be maintained during an A ↔ B transition and its collapse may well be a key factor in conferring cooperativity on this transition. The two polynucleotide strands are much further apart in the A conformation than in either the B or D forms. This feature can be related to the fact that both B and D are favoured over the A form by the presence of excess ions in the fibre. This effect is most noticeable for the D conformation, which exhibits a particularly narrow 'small' groove (figure 1). Since the ions providing the additional shielding to allow this can be expected to be hydrated, this effect can be related to the localized water in the neutron-diffraction analysis being concentrated in this groove. This is in marked contrast to the A form where the surface against which the central column of water is located is part of the 'large' groove of the double helix.

The changes observed in neutron fibre diffraction from the crystalline B conformation of natural 'random sequence' DNA when the H_2O surrounding the DNA was replaced by D_2O are illustrated in figure 12.

The definition of the patterns in figure 12 compared with those in figure 7 reflects improvements, since the initial studies on the D conformation, in specimen preparation, control of fibre hydration and strategies for recording and processing neutron diffraction data.

The data recorded to date in neutron fibre-diffraction studies of DNA have been primarily at relative humidities

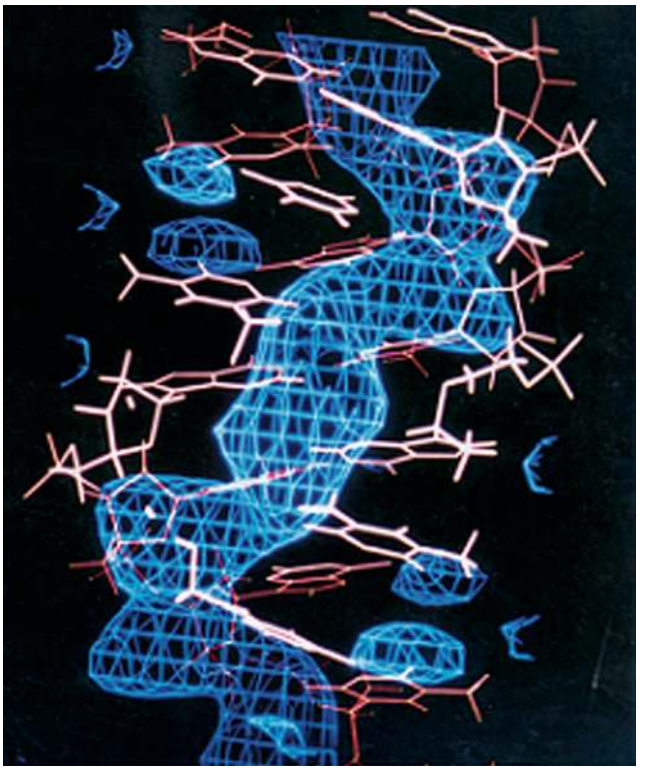

Figure 8. Fourier difference map showing localized water in the minor groove of the D conformation of DNA.

chosen to optimally favour a particular conformation. An approach to recording data from intermediate stages during the D ↔ B transition using a specially designed humidity control system (Shotton *et al.* 1998*b*) is illustrated in figure 13.

Further developments are underway at the ILL and EMBL in Grenoble including the upgrading of the single-crystal/fibre-diffraction instrument D19 as a high priority within the the ILL's *Millennium Programme*. The

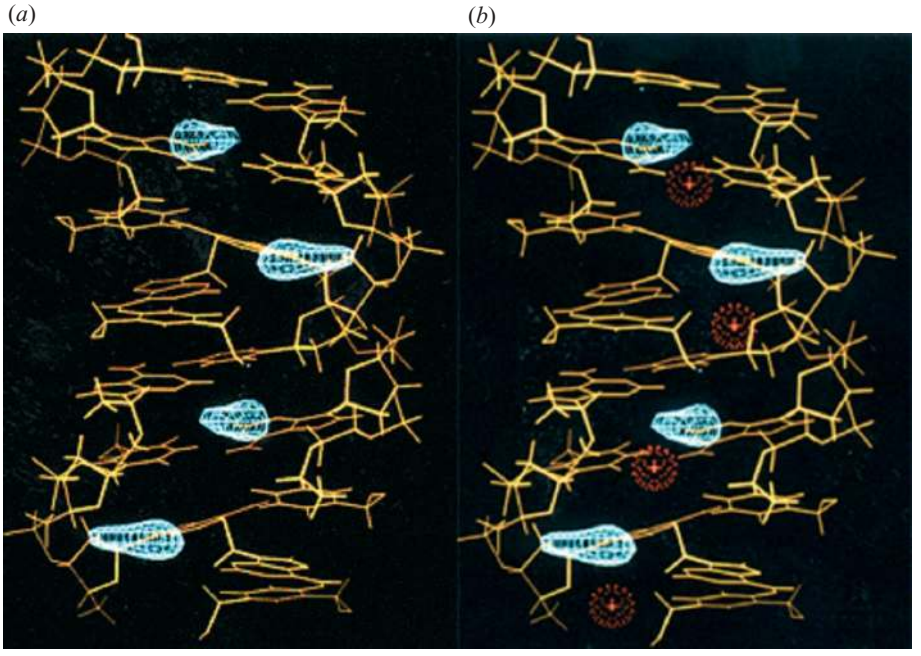

Figure 9. Fourier images showing the relationship between water and cations in the narrow minor groove of the D conformation of DNA. The phases used in both the X-ray and neutron Fourier difference syntheses were calculated from the model of the D conformation of DNA determined by Arnott *et al.* (1983).

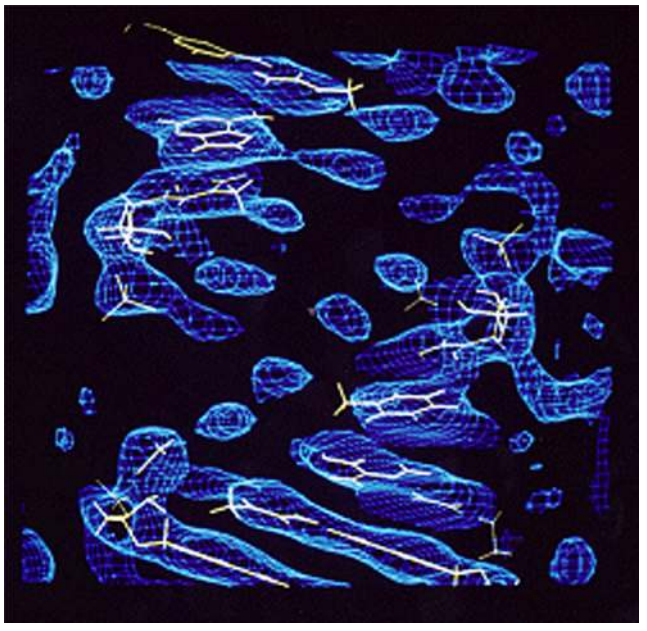

Figure 10. Section of a $(2F_o - F_c)$ Fourier map of the A conformation showing the water molecules bridging phosphate groups.

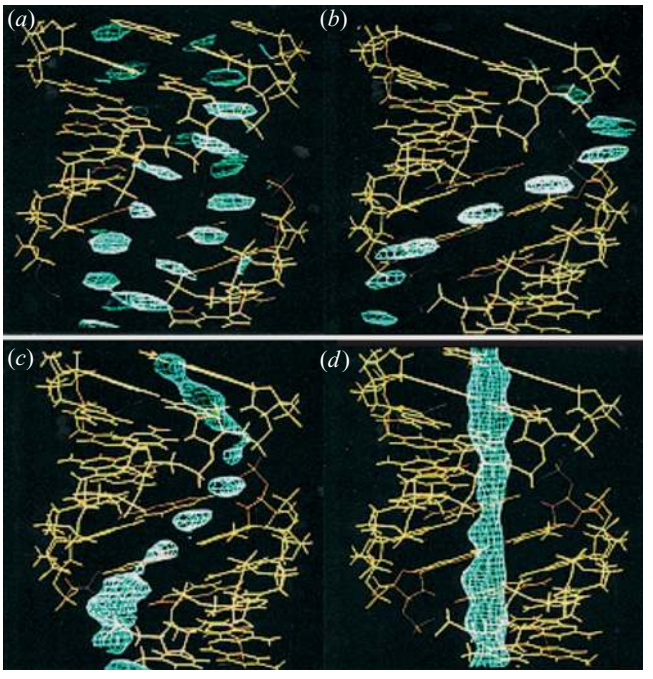

Figure 11. The results of a neutron-diffraction analysis showing four ordered water sites in A-DNA. (From Shotton *et al.* (1998*a*).)

refurbished instrument, which is planned to be available from 2005, incorporates a new detector, which will produce a gain of approximately 25 in solid angle (Forsyth *et al.* 2001). This will allow data to be collected on acceptable time-scales from smaller specimens and also offers the prospect of substantially improved resolution in time-resolved studies. The advantages of using deuterated DNA to reduce incoherent background scatter when recording neutron fibre-diffraction data were emphasized in previous sections. A facility has recently been established in Grenoble with support from the EPSRC and the EU for producing selectively and non-selectively deuterated material which will not only allow incoherent background scattering to be reduced but also the localization through Fourier difference imaging of specifically deuterated groups (Forsyth *et al.* 2002).

5. CONCLUSIONS

Fibre diffraction, through the structural flexibility that the fibrous state can accommodate, has proved to be a

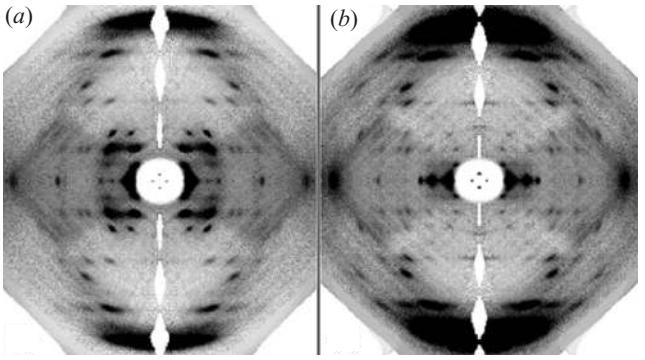

Figure 12. Neutron fibre-diffraction patterns recorded from (*a*) H_2O and (*b*) D_2O using the D19 diffractometer at the ILL.

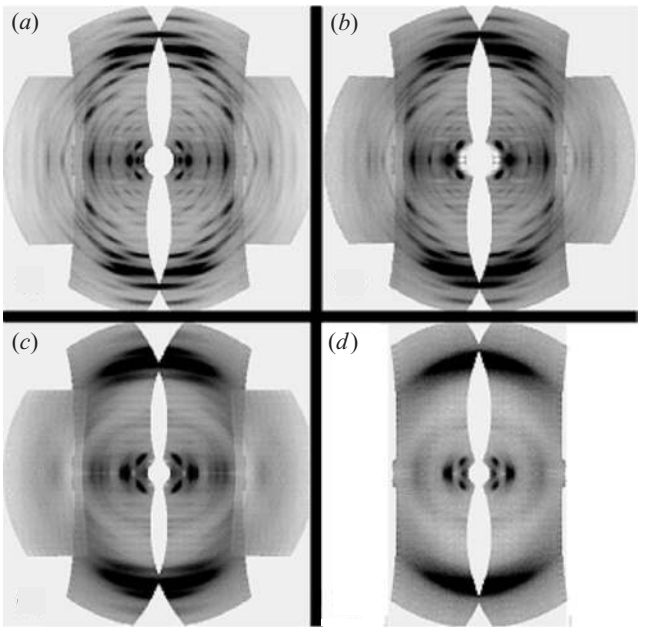

Figure 13. High-angle neutron fibre-diffraction patterns recorded during the transition from D (*a*) through intermediate structures (*b*,*c*) to B (*d*). The data were recorded using instrument D19 at the ILL.

powerful and versatile technique for identifying the conditions that favour particular conformations of the DNA double helix and that are responsible for inducing transitions between them. Although gradual distortions of particular conformations have been observed, conformations of the DNA double helix are generally well defined, with the end products of transitions being highly reproducible. In addition to demonstrating the humidity-driven character of these transitions, fibre diffraction has also allowed the role of ions in association with water to be investigated. However, it is important to emphasize that because diffraction data from DNA fibres are limited, at best, to a resolution of 2 Å such data cannot, in general, give structural information at atomic resolution. Information at this level, at least as far as non-hydrogen atoms are concerned, has, over the past two decades, become routinely available from structure determinations of oligonucleotide single crystals. Fibre-diffraction studies of the various conformations of DNA and single-crystal studies of oligonucleotides can be regarded as largely complementary since although single-crystal determinations can provide detailed information on atomic coordinates, there are limitations because a short length of oligonucleotide may be affected by 'end effects' and crystal-packing forces, which may result in local conformational distortions and the imposition of features in interactions with solvent and ions determined by crystal-packing forces. Further, unlike the situation within DNA fibres, the single-crystal environment is rarely able to accommodate significant conformational transitions. Despite limitations in the resolution of the diffraction data in both X-ray and neutron fibre-diffraction investigations, these techniques have provided unique information on stereochemical pathways in structural transitions in the DNA double helix and on interactions with water and ions. There can be no doubt that with the enhancements currently underway at both neutron and X-ray synchrotron sources the extent and level of detail of this information will continue to increase. However, in the context of this discussion meeting the crucial contribution made by fibre-diffraction techniques is not so much in providing stereochemical information about a particular interaction but in the more general inference to be drawn from the high degree of reproducibility and reversibility observed in humidity-driven structural transitions in the DNA double helix. This is that the stereochemistry of interactions between water and DNA must be highly specific. In the light of this and the unique stereochemistry of water, it is extremely difficult to envisage any substitute for water that would have the same degree of effectiveness as the medium in which the biochemical processes involving DNA takes place.

Work at the Daresbury Laboratory SRS, the ESRF and the ILL was supported by project grants from SERC and EPSRC and by allocations of beam time at these facilities.

REFERENCES

Arnott, S., Wilkins, M. H. F., Hamilton, L. D. & Langridge, R. 1965 Fourier synthesis of lithium DNA part III: Hoogsteen models. *J. Mol. Biol.* **11**, 391–402.

Arnott, S., Chandrasekaran, R., Birdsall, D. A., Leslie, A. G. W. & Ratliff, R. L. 1980 Left-handed DNA helices. *Nature* **283**, 743–745.

Arnott, S., Chandrasekaran, R., Puigjaner, L. C., Walker, J. K., Hall, I. H., Birdsall, D. L. & Ratliff, R. L. 1983 Wrinkled DNA. *Nucleic Acids Res.* **11**, 1457–1474.

Forsyth, V. T., Greenall, R. J., Hussain, R., Mahendrasingam, A., Nave, C., Pigram, W. J. & Fuller, W. 1986 X-ray high-angle fibre diffraction studies of nucleic acid structure using the Daresbury synchrotron radiation source. *Biochem. Soc. Trans.* **14**, 553–557.

Forsyth, V. T., Mahendrasingam, A., Pigram, W. J., Greenall, R. J., Bellamy, K. A., Fuller, W. & Mason, S. A. 1989 Neutron fibre diffraction studies of DNA hydration. *Int. J. Biol. Macromol.* **11**, 236–240.

Forsyth, V. T., Mahendrasingam, A., Langan, P., Pigram, W. J., Stevens, E. D., Al-Hayalee, Y., Bellamy, K. A., Greenall, R. J., Mason, S. A. & Fuller, W. 1990 X-ray and neutron high-angle fibre diffraction studies in the determination of hydration and ionic structure around DNA. In *Neutron and X-ray scattering: complementary techniques* (ed. M. C. Fairbanks, A. N. North & R. J. Newport), pp. 237–248. Bristol, UK: IOP Publishing Ltd.

Forsyth, V. T., Shotton, M. W., Ye, H., Pope, L. H., Boote, C., Langan, P. & Denny, R. C. 1998 Applications of instrument D19 at the Institut Laue-Langevin for the study of polymer systems by neutron diffraction. *Fibre Diffract. Rev.* **7**, 17–24.

Forsyth, V. T., Mason, S. A., Howard, J. A. K., Davidson, M. G., Fuller, W. & Myles, D. A. A. 2001 A fast new diffractometer for chemical crystallography, small proteins and fibre diffraction. *Neutron News* **12**, 20–25.

Forsyth, V. T., Myles, D. A. A., Timmins, P. A. & Hartlein, M. 2002 Possibilities for the exploitation of biological deuteration in neutron scattering. In *Opportunities for neutron scattering in the 3rd millennium* (ed. J. Dianoux), pp. 47–54. Grenoble: Institut Laue Langevin.

Franklin, R. E. & Gosling, R. G. 1953*a* The structure of sodium thymonucleate fibres. I. The influence of water content. *Acta Crystall.* **6**, 673–677.

Franklin, R. E. & Gosling, R. G. 1953*b* Molecular configuration in sodium thymonucleate. *Nature* **171**, 740–741.

Fuller, W., Wilkins, M. H. F., Wilson, H. R., Hamilton, L. D. & Arnott, S. 1965 The molecular configuration of deoxyribonucleic acid. IV. X-ray diffraction study of the A form. *J. Mol. Biol.* **12**, 60–80.

Fuller, W., Forsyth, V. T., Mahendrasingam, A., Pigram, W. J., Greenall, R. J., Langan, P., Bellamy, K. A., Al-Hayalee, Y. & Mason, S. A. 1989 High-angle neutron fibre diffraction studies of the distribution of water around the D form of DNA. *Physica* B **156/157**, 468–470.

Langan, P., Forsyth, V. T., Mahendrasingam, A., Pigram, W. J., Fuller, W. & Mason, S. A. 1992 A high-angle neutron fibre diffraction study of the hydration of the A conformation of the DNA double helix. *J. Biomol. Struct. Dynam.* **10**, 489–503.

Langridge, R., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F. & Hamilton, L. D. 1960*a* The molecular configuration of deoxyribonucleic acid. I. X-ray diffraction study of a crystalline form of the lithium salt. *J. Mol. Biol.* **2**, 19–37.

Langridge, R., Marvin, D. A., Seeds, W. E., Wilson, H. R., Hooper, C. W., Wilkins, M. H. F. & Hamilton, L. D. 1960*b* The molecular configuration of deoxyribonucleic acid. II. Molecular models and their Fourier transforms. *J. Mol. Biol.* **2**, 38–64.

Mahendrasingam, A., Forsyth, V. T., Hussain, R., Greenall, R. J., Pigram, W. J. & Fuller, W. 1986 Time-resolved X-ray diffraction studies of the B to D structural transition in the DNA double helix. *Science* **233**, 195–197.

Mahendrasingam, A., Denny, R. C., Forsyth, V. T., Greenall, R. J., Pigram, W. J., Papiz, M. Z. & Fuller, W. 1990 Time-resolved X-ray fibre diffraction studies of the S to B conformational transition in the poly[d(G–C)].poly[d(G–C)] double helix. In *Neutron and X-ray scattering: complementary techniques* (ed. M. C. Fairbanks, A. N. North & R. J. Newport), pp. 225–236. Bristol, UK: IOP Publishing Ltd.

Marvin, D. A. 1960 X-ray diffraction studies of nucleic acid. PhD thesis, University of London, UK.

Marvin, D. A., Spencer, M., Wilkins, M. H. F. & Hamilton, L. D. 1961 The molecular configuration of deoxyribonucleic acid. III. X-ray diffraction study of the C form of the lithium salt. *J. Mol. Biol.* **3**, 547–565.

Marvin, D. A., Wilkins, M. H. F. & Hamilton, L. D. 1966 Application of Fourier synthesis techniques to low-resolution fibre diffraction data: preliminary study of deoxyribonucleic acid. *Acta Crystall.* **20**, 663–669.

Pope, L. H., Shotton, M. W., Forsyth, V. T., Hughes, D., Denny, R. C. & Fuller, W. 1998 Structural polymorphism in a tubercidin analogue of the DNA double-helix. *Biophys. Chem.* **70**, 161–172.

Shotton, M. W., Pope, L. H., Forsyth, V. T., Langan, P., Denny, R. C., Giesen, U., Dauvergne, M.-T. & Fuller, W. 1997 A high-angle neutron fibre diffraction study of the hydration of deuterated A-DNA. *Biophys. Chem.* **69**, 85–96.

Shotton, M. W., Pope, L. H., Forsyth, V. T., Langan, P., Grimm, H., Rupprecht, A., Denny, R. C. & Fuller, W. 1998*a* A high-angle neutron fibre diffraction study of the hydration of B-DNA. *Physica* B **241–243**, 1166–1168.

Shotton, M. W., Pope, L. H., Forsyth, V. T., Denny, R. C., Archer, J., Langan, P., Ye, H. & Boote, C. 1998*b* New developments in instrumentation for fibre diffraction experiments. *J. Appl. Crystall.* **31**, 758–766.

Squire, J. (and 14 others) 2003 New CCP13 software and the strategy behind further developments: stripping and modelling of fibre diffraction data. *Fibre Diffract. Rev.* **11**, 7–19.

Wang, A. H. J., Quigley, G. L., Kolpak, F. J., Crawford, J., van Boom, J. H., van der Marel, G. & Rich, A. 1979 Molecular structure of a left-handed fragment at atomic resolution. *Nature* **282**, 680–686.

Watson, J. D. 1961 *The double helix: a personal account of the discovery of the structure of DNA*. New York: Athenaeum.

Watson, J. D. & Crick, F. H. C. 1953 Molecular structure of nucleic acids. *Nature* **171**, 737–738.

Wilkins, M. H. F., Stokes, A. R. & Wilson, H. R. 1953 Molecular structure of deoxypentose nucleic acids. *Nature* **171**, 738–740.

Discussion

P. Ball (*Nature, London, UK*). Is the state of DNA in fibres really comparable to that *in vivo*? You have these beautifully straight helices, but in the cell the molecule is very tightly wound around histones; in order to function (e.g. to be transcribed), it must be pulled away from the protein surface, the helix is greatly distorted, and pinning at each end will inhibit conformational changes. So, should we not perhaps assess the role of water in DNA interactions more in terms of the nature of chromatin hydration?

W. Fuller. There seems to be little reservation about the prevalence of B-DNA occurring *in vivo*, although in various situations (e.g. around histones) the molecule as a whole must have to bend. Richmond & Davey (2003) have determined the structure of DNA in single crystals of nucleosome core particles. They conclude that the DNA is predominantly in the B form with local distortions and irregularities, which facilitate its superhelical path in the nucleosome. More generally, although biological macromolecules typically have the capacity to undergo local distortions, the structures they commonly assume are relatively robust, persisting in a wide variety of environments. Where—as for DNA—there is polymorphism, structural transitions are generally between well-defined conformations and are promoted by specific changes in the macromolecule's environment. It is reasonable to expect that evolutionary pressures have favoured macromolecules with well-defined structures and that where opportunities for structural transitions exist they have been exploited in biological function. For DNA, the whole issue of the significance of the different conformations is becoming less controversial, for example the occurrence of Z-DNA binding proteins.

B. Halle (*Department of Biophysical Chemistry, Lund University, Lund, Sweden*). In your fibre-diffraction structure of D-form DNA, you showed a string of alternating water molecules and counterions along the very narrow minor groove. Can you say how deeply these species

penetrate the groove; that is, do they bridge the phosphate groups or do they coordinate the nucleotide bases?

W. Fuller. The water and cation peaks in figure 9*b* are in positions consistent with water molecules and ions interacting with each other and with base edge atoms. Other peaks in the Fourier difference maps are consistent with ions and water molecules coordinated with DNA phosphate groups.

B. Halle. Your finding of a central core of water molecules in A-form DNA is intriguing. Are these water molecules within hydrogen-bonding distance of external water molecules? Have they also been identified in single-crystal structures of A-DNA oligonucleotides?

W. Fuller. The 'central core' of water is considered in more detail in Shotton *et al.* (1997) where it is identified as a continuous core of density running along the helix axis in front of the bases in the major groove. This feature can be identified with a water site observed in the single-crystal study by Dock-Bregeon *et al.* (1989) of the oligonucleotide $[U(UA)_6A]_2$. This RNA analogue has an A-type conformation and the water site is located within possible hydrogen-bonding distance of adenine NH_2, thymine O_4, cytosine NH_2 and guanine O_6 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.

B. Halle. Do you have sufficient resolution to discriminate by neutron diffraction between water molecules and sodium ions in the minor groove of DNA?

W. Fuller. The neutron coherent scattering lengths for H, O, D and Na are −3.7409, 5.805, 6.674 and 3.63, respectively. At the resolution of these fibre-diffraction studies (*ca*. 3 Å), it is not possible to distinguish between the individual atoms within a water molecule. The presence of H in water (with its negative scattering length) tends to make H_2O essentially self-cancelling at this resolution. Sodium can therefore be expected to be distinguishable from H_2O in difference Fourier maps. However, this is not the main issue, since we are working in a situation where we are exploiting isotopic replacement of H_2O by D_2O, which at this resolution would give D_2O an overall scattering length $((6.674 \times 2) + 5.805)/3.63$ times greater than that of sodium.

A. Kornyshev (*Imperial College London, London, UK*). Regarding DNA fibres, hydration also affects the DNA–DNA interaction through the ordering of water layers around the opposing DNA.

W. Fuller. This is an important point and is reflected in the fact that in crystalline fibres there is a strong correlation between the symmetry and geometry of the crystal lattice assumed for a particular DNA conformation and the helical parameters of that conformation.

Additional references

Dock-Bregeon, A. C., Chevrier, B., Podjarny, A., Johnson, J., De Bear, J. S., Gough, G. R., Gilham, P. T. & Moras, D. 1989 Crystallographic structure of an RNA helix $[U(UA)_6A]_2$. *J. Mol. Biol.* **209**, 459–474.

Richmond, T. J. & Davey, C. A. 2003 The structure of DNA in the nucleosome core. *Nature* **423**, 145.