Reports

Time-Resolved X-ray Diffraction Studies of the B ⇄ D Structural Transition in the DNA Double Helix

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Because of the relation between topology and function, there has been much interest in the structural transitions of the various conformations of DNA polymers. The x-ray fiber diffraction analysis system at the Daresbury Synchrotron Radiation Source was used to study the reversible transition between the B and D forms of the synthetic DNA poly[d(A-T)] · poly[d(A-T)]. The gradual progression of conformations between these two forms indicates that the DNA double helix does not undergo a change of handedness during this transition.

NATURAL DNA CAN ADOPT THE A, B, and C conformations (*1–3*) depending on its hydration and the ionic content of its environment. Two-stranded synthetic DNA polymers having regular repetitive base sequences can in addition adopt variants of the B form or even completely novel forms such as D for poly[d(A-T)] · poly[d(A-T)] (*4*) and poly[d(I-C)] · poly[d(I-C)] (*5*) and S for poly[d(G-C)] · poly[d(G-C)] (*6*). In some of these conformations a dinucleotide rather than a mononucleotide constitutes the structural repeating unit, and some have a left-handed helical structure. Such conformations, even if they persist in only limited regions of natural DNA, could form distinctive recognition sites, and left-handed segments would have profound implications for the topological unwinding problem. It is now known that regions of the S (or Z) form do exist in natural DNA (*7, 8*), and regions of DNA rich in adenine and thymine residues have been described as centers for controlling the transcription of genetic information. Detailed in vitro studies of the conformational properties of relatively simple oligonucleotides and polynucleotides can therefore be crucial to an understanding of the much more complex situation in natural DNA.

Both the helix sense and the number of nucleotide pairs per helical pitch in the D conformation have been the subject of prolonged controversy. Mitsui *et al.* (*5*) suggested that poly[d(I-C)] · poly[d(I-C)] was left-handed and eightfold. Arnott *et al.* (*9–11*) have reported more extensive diffraction data and described thoroughly refined right-handed, eightfold models for both poly[d(A-T)] · poly[d(A-T)] and poly[d(I-C)] · poly[d(I-C)]. These structures exhibit small conformational differences between the purine and pyrimidine nucleotides and are described as "wrinkled." In contrast Sasisekharan and co-workers (*12, 13*) have claimed, on the basis of energy calculations and fiber diffraction analysis, that equally acceptable left- and right-handed eightfold models can be built for both poly[d(A-T)] · poly[d(A-T)] and poly[d(I-C)] · poly[d(I-C)]. Drew and Dickerson (*14*) have proposed a completely different type of model to account for the diffraction from poly[d(A-T)] · poly[d(A-T)]. This model is left-handed and sevenfold with Hoogsteen base pairing between the two polynucleotide chains rather than the Watson-Crick pairing in all the other models summarized above.

We have described the conditions for observing the semicrystalline B and crystalline D conformations of poly[d(A-T)] · poly[d(A-T)] in oriented fibers and for inducing a reversible transition between these two forms by varying the relative humidity of the fiber environment (*15*). We have combined the very high brightness of the Daresbury Laboratory Synchrotron Radiation Source (SRS) (*16*) with this capability for precisely controlling the B ⇄ D transition to record the detailed variation of the x-ray fiber diffraction pattern during this transition.

The time course of the experiment is shown schematically in Fig. 1. During the 17-hour period approximately 100 diffraction patterns were recorded, and the fiber underwent four D → B → D transitions. Transition times were typically about an hour, which allowed a sequence of eight to ten diffraction patterns per transition to be recorded. The sequence of patterns corresponding to a single transition represents only a fairly coarse time resolution, but by varying the transition rate and pooling the data from different transitions one obtains a much more complete picture. The diffraction patterns in Figs. 2 and 3 show representative stages from these transition sequences.

The degree of crystallinity and orientation in the initial D pattern (Fig. 2a) is significantly greater than reported previously for this form (*4, 9, 10*). Two of the more intense reflections in this pattern are identified as I and II. Their intensity in the other patterns in Fig. 2 is an indicator of the degree to which the D form is present at a particular relative humidity. The D form is crystalline with lattice parameters that do not vary significantly with the relative humidity of the fiber environment. Over all the patterns in which the D form was observed, reflection I has an average spacing of 17.5 ± 0.2 Å and the average helical pitch calculated from measurements on reflection II is 24.3 ± 0.3 Å (errors are standard deviation, σ). In contrast, the spacings associated with the reflections identified as III and IV in Fig. 2c exhibit a smooth variation with the relative humidity of the fiber environment and a high degree of reproducibility between successive transitions (Fig. 1). Furthermore, there is a clear correlation between the intermolecular separation and the helical pitch. The variation in the spacings of reflections III and IV is large enough to be readily seen by eye (Fig. 2). Reflection II in Fig. 2a has acquired a very close neighbor, reflection IV in Fig. 2c. In the sequence Fig. 2, b to f, the reflections characteristic of the D form become progressively weaker, and reflection IV moves progressively in an approximately radial direction toward the center of the diffraction pattern corresponding to an increase in both the helical pitch and the intermolecular separation in the intermediate semicrystalline form. In Fig. 2f reflection IV has assumed the position of the first-layer line reflection characteristic of the semicrystalline B form with a pitch of 33.5 Å. Associated with this gradual increase in the helical pitch from 24 to 34 Å there is an increase in the intensity of the crosslike distribution characteristic of the semicrystalline B form of DNA.

The sequence of diffraction patterns in Fig. 3, recorded while the relative humidity of the fiber environment was reduced from 98 to 55%, exhibits a variation in the spacings of reflections III and IV very similar to that in Fig. 2. However, the changes in the

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continuous diffraction are more complex. Fig. 3a is a well-defined semicrystalline B pattern in which the continuous diffraction in the center of the pattern is concentrated on the second- and third-layer lines. As the relative humidity is decreased the second- and third-layer lines become less sharp, and continuous diffraction is observed in the region between them (Fig. 3, a and b). The overall distribution of continuous diffraction in Fig. 3d is very similar to that in Fig. 3c but, within the regions where this diffraction is strong, sharp reflections can also be seen. These reflections have spacings characteristic of the fully crystalline D pattern, as can be seen from a comparison with Fig. 3f. Figure 3e represents an intermediate stage in this progression toward the crystalline D.

For a fiber in the crystalline D form, raising the relative humidity results in a loss of crystallinity in some regions of the fiber. Therefore, while the regions unaffected by this increase in water content still give crystalline D diffraction, others give diffraction indicating that the molecules are packed in a semicrystalline array. Reflections III and IV in Fig. 3, b to e, are the two best defined reflections in the semicrystalline diffraction; they indicate regular packing of the molecules and a well-defined helical pitch. Although the radial broadening of reflection IV in these patterns is rather greater than that of reflection II, it is still sufficiently sharp to indicate that all the molecules in the semicrystalline regions of the fiber had a similar helical pitch, which remained essentially constant during the 5 minutes or so while the diffraction was recorded.

The correlation between helical pitch and intermolecular separation indicated in Fig. 1 further supports the view that the degree of hydration and the helical pitch of molecules in the semicrystalline region are correlated. The molecule does not assume a B conformation with its characteristic 34-Å pitch immediately on being released from the constraints of the crystalline D lattice. Rather it appears that, for such a relatively unconstrained molecule, a particular degree of hydration stabilizes a conformation with a particular pitch. An increase in that degree of hydration results in a corresponding increase in pitch until the B conformation is assumed. Once all the molecules are in the semicrystalline B conformation, the intermolecular separation continues to increase as the degree of hydration increases but with little if any change in conformation as is indicated by the constant helical pitch.

The changes in helical pitch and intermolecular separation associated with reducing the relative humidity of the fiber environment follow a pattern of variation similar to that which occurs as the relative humidity is raised (Fig. 1). Furthermore, the pitches of the intermediate conformations observed during the B ⇄ D transition are not limited to particular ranges with obvious forbidden regions. In addition, the values observed for the pitch of the intermediate conformations always lie between the standard values for the D and B conformations, that is, between 24 and 34 Å.

When the pitch associated with reflection IV is in the region of, say, 31 to 34 Å (Fig. 2, c, d, and e), the continuous diffraction has the crosslike appearance on the first, second, and third layer lines characteristic of the B conformation (Fig. 2f), whereas it has an overall intensity distribution similar to that of the D conformation when the pitch is in the region of 24 to 27 Å (Fig. 3, c, d, and e). As might be expected, the similarity to the B intensity distribution is reduced as the pitch falls from 34 Å, whereas the similarity to the D distribution is reduced as the pitch rises from 24 Å. The reduction in the degree of identity with either the B or D intensity distributions is associated with the layer lines in the semicrystalline region of the diffraction pattern as they become less well defined and with the appearance of continuous diffraction between them (Fig. 3, b and c).

The above demonstration that in the D → B transition the helical pitch of the molecule gradually increases from 24 to 34 Å and in the B → D transition gradually decreases from 34 to 24 Å is relevant to both the current interest in structural transitions between left- and right-handed conformations of the DNA double helix (*17–20*) and the controversy over the handedness of the D form (*9, 11–14*). The only physically plausible model that can account for the change in pitch during the B ⇄ D transition is one in which there is a gradual change in the helical conformation. In particular, a change in handedness of the helix is incompatible with such a gradual distortion.

This assertion may be illustrated by considering two types of model for a change in helix sense. The first of these is the rather obvious and natural transformation involving the untwisting of, say, a right-handed B helix followed by twisting in the opposite sense to yield a left-handed D helix. In such a transition the magnitude of the helical pitch would increase until it became effec-

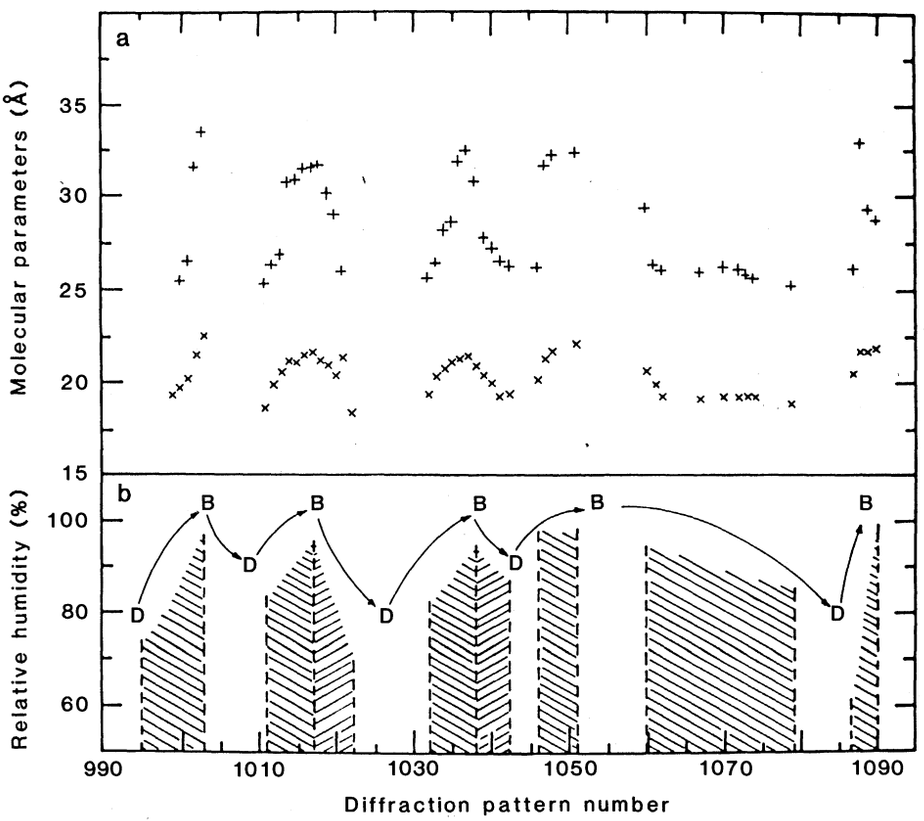

Fig. 1. (a) Variation in the lateral intermolecular spacing (×) and helical pitch (+) of the intermediate semicrystalline form as a function of relative humidity. (b) Variation of the relative humidity of the fiber environment and molecular conformation during the experiment. Approximately 100 diffraction patterns (numbers 994 through 1090) were recorded with exposures of 4 to 10 minutes, depending on the intensity from the SRS, over a period of 17 hours during which four reversible transitions between the crystalline D form and the semicrystalline B form were observed. In the gaps between the shaded regions the conformation was held constant in either the D or the B form.

tively infinite and would then progressively decrease to its final value. In contrast, there is no evidence from these studies that the pitch ever exceeded 34 Å. It should be emphasized that, had the pitch increased above 34 Å, no difficulty in observing its variation would have been expected. Such a variation was observed for the increase of the pitch of DNA after the binding of intercalating drugs (*21*). The second type of model for a change in handedness in the B ⇄ D transition is derived from that proposed by Wang *et al.* (*18*) from their studies of Z-DNA. Such a model involves the sequential breaking and reforming of the base pairs linking the two polynucleotide chains so that "regions of transition" consisting of a few base pairs can be imagined to travel along the length of the DNA double helix. If the B ⇄ D transition was described by this model, such a region would have a left-handed D segment on one side and a right-handed B segment on the other. On the basis of such a model, diffraction observed during the transition would consist of a mixture of the normal semicrystalline B and crystalline D diffraction patterns. This is clearly at variance with our observation of a gradual change in helical pitch as molecules change from B to D form or D to B form.

In this brief discussion we have not been able to consider every possible type of model of the B ⇄ D transition that involves a change in helix sense. However, the two models considered are not only the most obvious but also represent the two general types of model that might be proposed. Since neither of them can account for our x-ray diffraction observations, we conclude that (i) the helix sense does not change during the B ⇄ D transition in poly[d(A-T)] · poly[d(A-T)] and (ii) if, as is generally accepted, the B conformation of DNA is right-handed, then left-handed models for D-DNA, and in particular those proposed by Drew and Dickerson (*14*) and Sasisekharan and co-workers (*12*), cannot be correct.

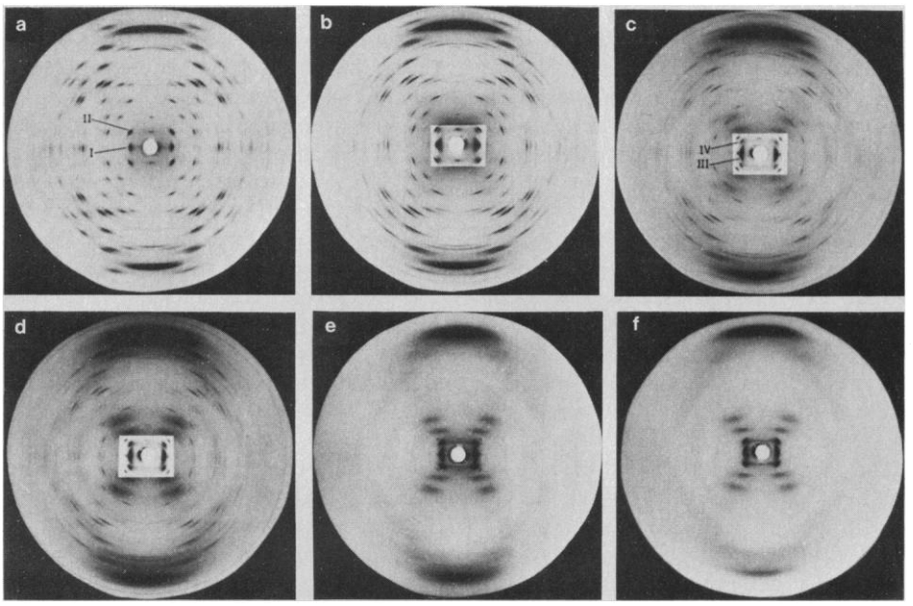

Fig. 2. Diffraction patterns illustrating typical stages in the D → B transition. In (a) the reflection marked I is related to the lateral intermolecular separation and that marked II to the helical pitch of molecules in the D form. In (c) the reflection marked III is related to the lateral intermolecular separation and that marked IV to the helical pitch of molecules in the semicrystalline intermediate form. The pattern numbers of the diffraction patterns (a through f) are 995, 1046, 1047, 1048, 1002, and 1003, respectively. In the reproduction of patterns (b), (c), and (d) diffraction in the central region of the pattern has been attenuated so that neighboring reflections are more easily resolved.

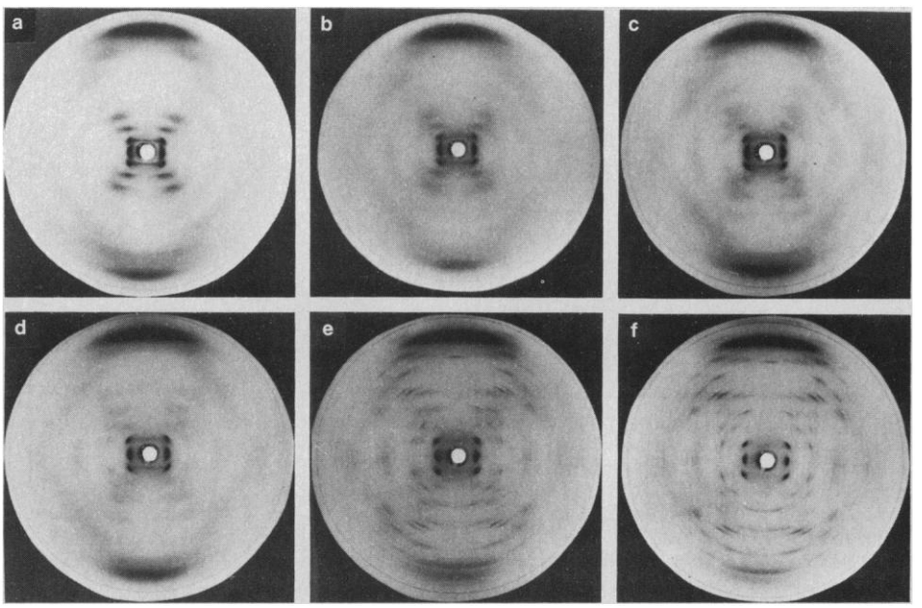

Fig. 3. Diffraction patterns illustrating typical stages in the B → D transition. The numbers of the patterns (a through f) are 1051, 1061, 1062, 1067, 1074, and 1084, respectively.

REFERENCES AND NOTES

1. W. Fuller, M. H. F. Wilkins, H. R. Wilson, L. D. Hamilton, *J. Mol. Biol.* **12**, 60 (1965).
2. R. Langridge *et al.*, *ibid.* **2**, 38 (1960).
3. D. A. Marvin, M. Spencer, M. H. F. Wilkins, L. D. Hamilton, *ibid.* **3**, 547 (1961).
4. D. R. Davies and R. L. Baldwin, *ibid.* **6**, 251 (1963).
5. Y. Mitsui *et al.*, *Nature (London)* **228**, 1166 (1970).
6. S. Arnott, R. Chandrasekaran, D. L. Birdsall, A. G. W. Leslie, R. L. Ratliff, *ibid.* **283**, 743 (1980).
7. A. Nordheim and A. Rich, *ibid.* **303**, 674 (1983).
8. A. Nordheim *et al.*, *ibid.* **294**, 417 (1981).
9. S. Arnott, R. Chandrasekaran, D. W. L. Hukins, P. J. C. Smith, L. Watts, *J. Mol. Biol.* **88**, 523 (1974).
10. A. G. M. Leslie, S. Arnott, R. Chandrasekaran, R. L. Ratliff, *ibid.* **143**, 49 (1980).
11. S. Arnott *et al.*, *Nucleic Acids Res.* **11**, 1457 (1983).
12. G. Gupta, M. Bansal, V. Sasisekharan, *Int. J. Biol. Macromolec.* **2**, 368 (1980).
13. N. Ramaswamy, M. Bansal, G. Gupta, V. Sasisekharan, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6109 (1982).
14. H. R. Drew and R. E. Dickerson, *EMBO J.* **1**, 663 (1982).
15. A. Mahendrasingam *et al.*, *Nature (London)* **301**, 535 (1983).
16. C. Nave *et al.*, *J. Appl. Crystallogr.* **18**, 396 (1985).
17. F. M. Pohl and T. M. Jovin, *J. Mol. Biol.* **67**, 375 (1972).
18. A. H.-J. Wang *et al.*, *Nature (London)* **282**, 680 (1979).
19. A. Mahendrasingam, W. J. Pigram, W. Fuller, J. Brahms, J. Vergne, *J. Mol. Biol.* **168**, 897 (1983).
20. A. Rich, A. Nordheim, A. H.-J. Wang, *Annu. Rev. Biochem.* **53**, 791 (1984).
21. W. J. Pigram, W. Fuller, L. D. Hamilton, *Nature (London) New Biol.* **235**, 17 (1972).

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