Fibre diffraction of lithium DNA shows structural variability and deviation from a regular helical structure for the B-form

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Recently, reports have appeared which show structural variations in B-DNA and indicate deviations from a uniform helical structure. We report for the first time that these indications are also present in the B-form fibre diffraction patterns for the lithium salt of natural DNA. We have used an improved method of controlling the salt concentration in the fibres. Our results are based on the appearance and disappearance of meridional reflections on different layer lines depending upon the salt.

Fiber diffraction *Lithium B-DNA* *Non-ideal helix* *Structural variation*

1. INTRODUCTION

Ever since the observation of the typical high humidity cross pattern in the fibre diffraction of natural DNA [1] and the development of the theory of diffraction of X-rays by helical structures [2], it has generally been accepted that the structure of DNA is represented by a double helix having 10 base pairs per turn. This form, designated the B-DNA, is the one that is believed to exist in vivo. For some years it has been suspected that the secondary structure of DNA may be sequence dependent and this means that natural DNA, which generally have a more or less random sequence of bases, would have different local structures along a molecule. However, until recently it was believed that a deviation of the structure from a regular helical one would be marginal, if any. Recently, a number of studies have been reported which clearly show that a (sequence-dependent) non-uniformity in the DNA structure is significant enough to be detected, both in solution and in the solid state. Sequence-dependent cleavage patterns by certain nucleases have shown the existence of a structural polymorphism in solution [3,4], within the B-form. Crystal structure data on B-form oligomers have established that there can be considerable variation in local structural parameters: for example, the dodecamer d(CGCGAATTCGCG) structure [5] has values of unit twist ranging from 27° to 41° in contrast to the ideal value of 36°, and is very much dependent on the base sequence [5–7]. Structural variations in the fibrous state have also been detected by ^{31}P NMR studies on B-form fibres [8–10] which indicate considerable heterogeneity in the conformation of the phosphate backbone. We show that evidence pointing to a non-uniform structure for natural DNA is also provided by X-ray diffraction of Li-DNA fibres in the B-form. All these facts suggest the presence of a variety of conformations all belonging to the wide class called the 'B-form', and interconversion from one conformation to another within this range is not uncommon.

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Abbreviation: r.h., relative humidity

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2. MATERIALS AND METHODS

Sodium salt of calf thymus DNA (Sigma) was dissolved in deionised water in a concentration of 3–4 mg/ml and LiCl solution was added to make the LiCl concentration 0.1 M. To obtain the required salt-DNA ratio in the DNA solution (this ratio is denoted by Δ = ratio of Li^+ concentration to the concentration of DNA, the latter being measured in units of DNA phosphates), this was dialysed against a known concentration of LiCl solution for 36 h with 6 changes. The resultant Li^+ concentration in the DNA solution was calculated using the Donnan equilibrium principle. Details of this procedure are being published elsewhere [11]. After dialysis, the solution was condensed to form a thick gel. Fibres were drawn by allowing a drop of this gel to dry on parafilm-coated tips of paper clips suitably cut, so that the length of a dried fibre was 3–4 mm. Photographs were taken with a Ni-filtered Cu-K_α beam. To obtain a diffraction pattern at a particular r.h., the dried fibre was detached from the paper clip and enclosed in a Lindemann capillary tube (diameter 5 mm) together with a suitable saturated salt solution giving the required aqueous tension.

3. RESULTS AND DISCUSSION

It is known that the nature and concentration of the counterion, and also the relative humidity in the neighbourhood of the fibre, play an important role in determining the polymorphic form (viz., A,B,C) and crystallinity of the diffraction pattern for natural DNA [12]. While variation of r.h. is easily achieved, contemporary methods of controlling the counterion concentration in the fibre are far from perfect, and this has led to a subjectivity and poor reproducibility in obtaining good fibres. For the first time, we have obtained fibres at known salt (Li) concentrations, using the method described in the previous section for controlling the salt content in the gel. This has resulted in a high reproducibility of our experiments, and in unusual appearance and disappearance of meridional reflections in the diffraction patterns, depending upon the amount of salt. Our results do not support the concept of natural DNA having a regular helical structure in the solid state – based on which all model building studies have so far been made.

Diffraction patterns were obtained with lithium as the counterion, which is known to give the highly crystalline B-form [12]. We find that crystalline B-patterns are obtained at moderate (66%) r.h. for $\Delta = 1.2-1.9$, and within this region, the salt range $\Delta = 1.4-1.6$ is the one giving the best crystallinity. For this reason, we shall confine our attention chiefly to this region of salt. For this range, meridional reflections are observed on the fourth and sixth layer lines. The intensities of these reflections are found to depend on Δ. In addition, the strong tenth layer meridional streak is seen to consist of two or three spots, again depending on Δ, and in the former case the meridian on the tenth layer is absent. These observations are detailed below.

3.1. *Reflection 004*

The 004 reflection is observed as a weak diffraction spot throughout the range $\Delta = 1.4-1.6$ (see fig.1). On further increasing the salt ($\Delta = 1.9$), it was not seen (fig.2).

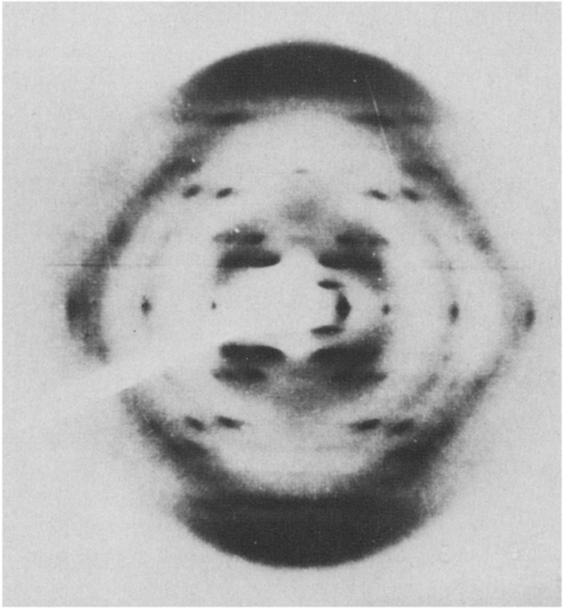

Fig.1. B-type diffraction patterns for Li-fibres appropriately tilted. $\Delta = 1.6$, showing the 006 and 004 reflections. For $\Delta = 1.4$, these are weaker (not shown).

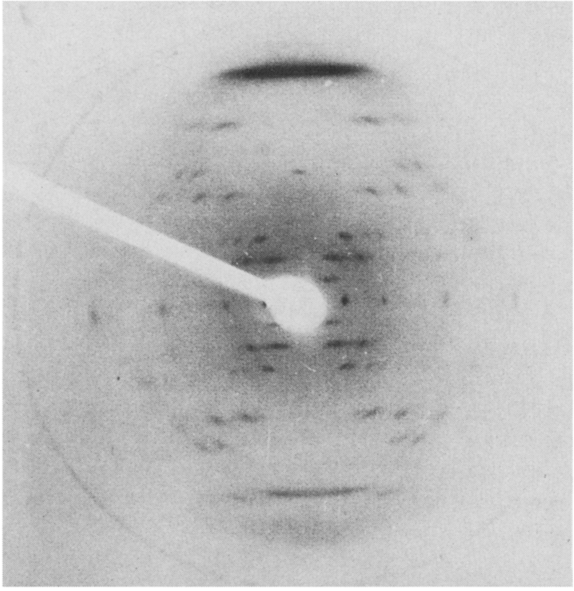

Fig.2. B-type diffraction patterns for Li-fibres appropriately tilted. $\Delta = 1.9$, with highly intense 006 reflection. LiCl ring appears at this high value of Δ, which is the limiting one for obtaining crystalline patterns.

3.2. *Reflection 006*

This reflection is observed as a sharp, strong meridian in both the pictures (fig.1,2). A comparison shows that its intensity increases with salt, and it is very intense at $\Delta = 1.9$ (fig.2). It is of comparable intensity to the other reflections, as evident from fig.2.

3.3. *Reflection 0010*

For $\Delta = 1.4$, an appropriately tilted fibre shows the tenth layer meridional streak to be devoid of any meridian and to consist of just two off-meridional spots, as seen in fig.3a. However, for $\Delta = 1.6$, such a photograph shows a meridional reflection together with the two off-meridional ones on the tenth layer (fig.3b). Low exposure times were given to record these finer details, since the meridional streak usually appears as a highly intense reflection, broad and fudgy.

3.4. *Implications*

Although the 004 and 006 reflections have earlier been observed in the crystalline B-pattern of

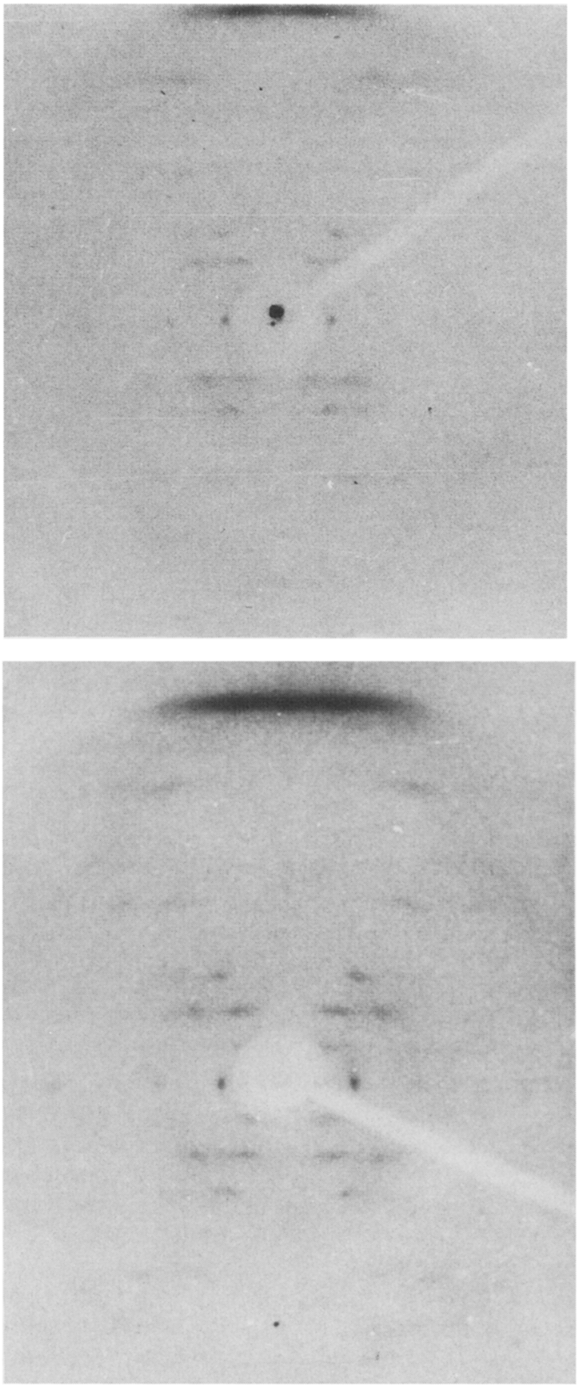

Fig.3. B-type diffraction patterns for Li-fibres appropriately tilted. The tenth layer meridional streak for (a, top) $\Delta = 1.4$ and (b, bottom) $\Delta = 1.6$. Note the structure of this streak for the two salts: two off-meridional reflections for $\Delta = 1.4$ and a meridional and two off-meridional reflections for $\Delta = 1.6$.

Li-DNA [13], the above-mentioned features on the tenth layer have not been reported so far. In the earlier report [13] however, the 004 and 006 reflections were obtained as weak diffraction spots, and were ignored. On the other hand, we obtain the 006 reflection with a strong intensity for a particular Δ value (as stated above). Its appearance (nature, shape, intensity, etc.) makes it indistinguishable from other reflections in the fibre pattern. The reproducibility and high intensity of the 006 reflection suggest that it cannot be ignored as due to some non-helical arrangement of water molecules and/or hydrated cations, as was done in [14]. Similarly, the variation of intensity on the tenth layer line cannot be ignored.

The above observations are not compatible with a helical structure for DNA. These suggest that deviations from an ideal helix are indeed present in the structure and are substantial enough to show up in the fibre pattern. This fact has actually been substantiated in an earlier report [14] where 'pairs of reflections having intensity ratios which are inconsistent with a perfectly helical structure' had been observed. However, these have been ignored simply because only helical structures were considered.

Further, it may be of interest to point out here that the earlier argument that 004 and 006 reflections could be due to solvent molecules leads to another inconsistency. Scattering from solvent should modify the intensity distribution for a helical structure throughout the reciprocal space, if at all. In that case the intensity of other comparable reflections also comes under question, and the subsequent refinement of DNA structures when computing the R-factor and deciding the favour of one model over another all become unreliable.

Thus, our results show certain features in the diffraction pattern for lithium B-DNA which point to deviations from the accepted helical structure. Some of these features (viz., presence of 004 and 006 reflections) had been observed before but were not considered seriously. It may be questioned that why then does the overall pattern show a helical cross? The invariance of the general intensity distribution indicates that the perturbation from a perfect helical structure is probably small. However, intensity measurements alone can tell how significant this perturbation could be. Our results only show that they are substantial enough to be detected in the fibre diffraction pattern. Also, the intensity variations of 006 and of the strong 0010 spots as a function of salt reveals a salt-dependent structural variability. These may have escaped the attention of earlier workers because of lack of knowledge in obtaining good fibres with exact amounts of salt.

4. CONCLUSION

Thus, an improved method of control of salt in Li-DNA fibres has revealed the correct salt region where the best crystalline B-pattern can be obtained with certainty. In this region a weak 004 and a strong 006 as well as strong or weak (absent) 0010 reflections appear. This evidence shows a structural variability and is inexplicable from the standpoint of an ideal helical structure. There are 3 distinct possible explanations for these observations: (i) nonhelical arrangement of hydrated Li^+ and/or water are responsible for the above phenomenon, as suggested in [14]. (ii) The 10 base-pair repeat unit has become distorted into two halves related by a screw axis parallel to the fibre axis, giving rise to meridional reflections on the even layer lines of which 004, 006 and 0010 are observed. Intensities of 002 and 008 might be too low to be recorded, and the intensities of all of them would depend upon the strength of the harmonics of the 17 Å periodicity of the axial projection. (iii) There exists a variability in the structure which is only an approximate helix.

Of the 3 alternatives, we reject the first for reasons stated in the previous section. Both the second and the third possibilities contain the idea that there exists a structural polymorphism in the B form which is influenced by salt concentration. Alternative (ii) implies that there would be a 17 Å repeat in the structure. This is not consistent with our measurements on the structural parameter n from layer line spacings in which a series of values different from 10 are obtained for different r.h. and Δ values (unpublished, see also [11]). Thus, taking into account the evidence pointing to a structural heterogeneity (especially the ^{31}P NMR work on lithium fibres which indicates nonuniform backbone conformation [9]), the third possibility is the most plausible one. Though it is believed that fibre diffraction gives only an average structure, exact control of conditions may have resulted in showing up the inhomogeneity and variability of the structure in our fibre patterns. The natural questions 'What does the structure then look like?' or 'How much exactly is the deviation from helicity?' remain unanswered at present. The dodecamer crystal structure [5] has already indicated the trend. Careful and extensive model building studies compared with X-ray data are necessary to provide answers to these questions.

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