

**B TO Z TRANSITION IN DNA FIBRE: THE QUESTION OF HANDEDNESS OF THE DUPLEX**

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## ABSTRACT

The B-form of poly(dG-dC) is assumed to be a right handed duplex because its diffraction pattern is the same as that of the B form of DNA. Poly(dG-dC), when in high salt, gives the Z pattern which has been interpreted as being due to a left handed zig-zag structure similar to that observed in single crystals of d(CpGpCpGpCpG) and d(CpGpCpG). We report here the observation that fibres of poly(dG-dC) drawn from 1:1 (v/v) water-ethanol also yield the Z pattern identical to the pattern obtained for the polymer high salt. Surprisingly, the same fibre, which gives a B pattern initially, appears to undergo a transformation in the solid state such that its diffraction pattern changes to a final Z pattern after it has been equilibrated at room temperature (22° C) and humidity. This demonstrates that the handedness of the duplex in both forms of DNA should be the same since this transition from B pattern to Z pattern takes place in the solid state. The change of handedness of the duplex in the poly(dG-dC) fibre following prolonged annealing, as suggested by Arnott and co-workers, is incompatible with the observations reported here.

## INTRODUCTION

For more than two decades DNA was believed to exist only in the right handed double helical conformation<sup>1</sup>. The Watson-Crick B form of DNA, it has generally been assumed, is the one present in biological systems. However, recently, left handed zig-zag double helical structures have been observed in crystals of d(CpGpCpGpCpG)<sup>2</sup> and in the high salt form of d(CpGpCpG)<sup>3</sup>. Furthermore, Wing *et al.*<sup>4</sup>, have indicated from single crystal structure analysis of another deoxy oligo nucleotide, d(CGCGAATTCGCG), the presence of a complete turn of right-handed B DNA type double helix in the crystal.

Before any of these interesting single crystal data were available, Sasisekharan and co-workers had suggested, on the basis of stereochemical considerations, that DNA could exist in both right and left handed double helical conformations<sup>5-10</sup>. A stereochemical guideline was formulated by Sasisekharan and co-workers to investigate the conformational flexibility inherent in DNA. This flexibility led to the possibility of a variety of right and left handed DNA duplexes, some of which have subsequently been seen in single crystals. Sasisekharan and co-workers made use of this stereochemical guideline to probe molecular conformations of DNA with alternating purine-pyrimidine sequences<sup>10-13</sup>. It was observed that there could be a variety of sequence-specific and conformationally distinct right and left handed duplexes, and that any right handed fragment could be joined to any left handed fragment to give rise to a RL model with stable inverted stacking arrangement at the link<sup>15</sup>.

Poly(dG-dC) is the only polynucleotide known whose structure has been interpreted in terms of a right handed helical conformation under certain conditions

and a left handed helical conformation under certain other conditions on the basis of X-ray fibre diffraction and solution studies<sup>14</sup>. Pohl and Jovin were the first to show that this polymer in solution could undergo a cooperative transition by salt and ethanol<sup>14-15</sup>. They indicated that the conformation of the polymer in low salt is similar to that of the right handed B DNA, whereas its conformation in high salt is a double helix of unknown left-handed conformation. The crystal structures of DNA fragments, d(CpGpCpGpCpG)<sup>2</sup> and d(CpGPCpG)<sup>8</sup>, appear to have vindicated their hypothesis, namely, that the high salt form of poly(dG-dC) is a left handed duplex. Arnott *et al.*<sup>16</sup> have obtained diffraction patterns of poly(dG-dC) fibre with 4-6% retained sodium chloride and after prolonged annealing. The diffraction pattern obtained has been interpreted as being due to a left handed Z-structure similar to that observed in single crystal<sup>2,3</sup>, while the pattern of the low salt fibre was interpreted as right handed B DNA. In this paper, we present the results of X-ray fibre diffraction studies on fibres of poly(dG-dC) drawn from 1:1 (v/v) water-ethanol mixture. We show here that merely by a change of humidity and equilibrating in air, there is a change in the X-ray diffraction pattern of the poly(dG-dC) fibre from the characteristic cross pattern of B DNA to the pattern reported as Z DNA<sup>16</sup>. This interconversion under such mild experimental conditions and in the solid state, rules out the possibility of a change in handedness of the duplex in the fibre.

## MATERIALS AND METHODS

The polymer used in the present study was obtained from PL Biochemicals, Wisconsin, USA. The lyophilized polymer (0.5 mg) with minimum retained sodium chloride as indicated by the manufacturer was

dissolved in the minimum amount of 1: 1 (v/v) ethanol-water mixture to obtain a gel. Fibres were then drawn from this gel by allowing them to dry on U-shaped teflon coated wire at 4°C. These fibres were then used to obtain diffraction patterns. A flat plate camera with the specimen-to-film distance adjustable from 4 cms to 6 cms was used. The specimen-to-film distance was calibrated in each case with a quartz fibre of known spacings. Humidity was controlled by using saturated solutions of appropriate salts and by mounting the fibre in a Lindermann glass capillary with the salt solution at one end of the sealed capillary.

### RESULTS

Figure 1 *a-d* shows the X-ray diffraction patterns of such a poly(dG-dC) fibre at different intervals of time drawn from 1 : 1 (v/v) water-ethanol mixture at 4° C. Diffraction patterns were recorded in a flat plate camera using  $\text{CuK}_\alpha$  radiation with specimen 10 film distance calibrated using a quartz fibre of known spacings.



Fig. 1a. Diffraction pattern of a fibre taken at 40% humidity (22°C) immediately after the fibre was dry – Note the characteristic B pattern.



FIG. 1 *b*. Diffraction pattern of the same fibre taken immediately after the first picture was taken. All the ten layer lines are seen. Meridional and near meridional reflections are observed as streaks.

As can be seen, there is a transition from the typical cross pattern of B DNA to the Z pattern. Fig. 1 *a* shows the picture taken at 40% humidity (22° C) immediately after the fibre was dry. It is readily seen that this pattern has all the characteristics of B DNA, Li-DNA at 66% rh<sup>17</sup>, Na-DNA at 92% rh<sup>17</sup> and poly(dG-dC) in B form<sup>16</sup>. This fibre quickly (6 hrs) underwent transition at 40% humidity to a form which gave an X-ray pattern shown in Fig.1b. Diffraction pattern of this type has not been reported so far for DNA or for any form of synthetic analogs of DNA. The pattern has, in addition to the features of B DNA, meridional or near meridional reflections on the various layer lines. All the ten layer lines are distinctly seen and meridional and near meridional reflections on the layer lines are observed as streaks. This pattern (1b) which has not yet been interpreted remains unaltered for a few hours (15-20 hrs) at room humidity.

Contrary to our expectation, on raising the humidity to 92%, this fibre did not revert and yield a B DNA pattern.

On the other hand, raising the humidity to 92% and then bringing it back in steps to 40% humidity resulted in the X-ray diffraction pattern shown in Fig. 1 *c*. This picture has both the characteristic cross-pattern due to B form as well as the meridional or near-meridional reflections of the Z pattern reported for poly(dO-dC) at high salt<sup>16</sup>. This unusual diffraction pattern was also obtained without going through the humidity cycle described above; a fibre which gave pattern 1 *b*. after equilibrating several hours at room humidity gave a diffraction pattern similar to the one shown in Fig. 1 *c*.

When the fibre was kept at room humidity for a few days and then studied, it gave the diffraction pattern shown in Fig. 1 *d*. This picture resembles very much the poly(dG-dC) pattern obtained at high salt<sup>16</sup>. The essential difference

between our Fig. 1 *d* and Fig. 1 *c* of Arnott et al.<sup>16</sup>, is that the latter is more crystalline than the former. The quality of the Z pattern did not improve any further by equilibrating the fibre for several days at a constant humidity

of 43%. No further attempt to improve the quality of the diffraction pattern was made because the primary purpose of the present investigation was to find out whether, in the solid state, a transformation could take place from the B pattern to the Z by the meridional reflections on the 6th, 12th and 13th layer lines and by non-meridional reflections on the 8th and 10th layer lines. The C-axis repeat is 43.2 Å, identical to that obtained by Arnott *et al*<sup>16</sup>. It is interesting to note that both 3.6 Å and 3.4 Å reflections are strong and the spacing of 6th layer line is double the 12th layer line (Fig. 1 *d*).

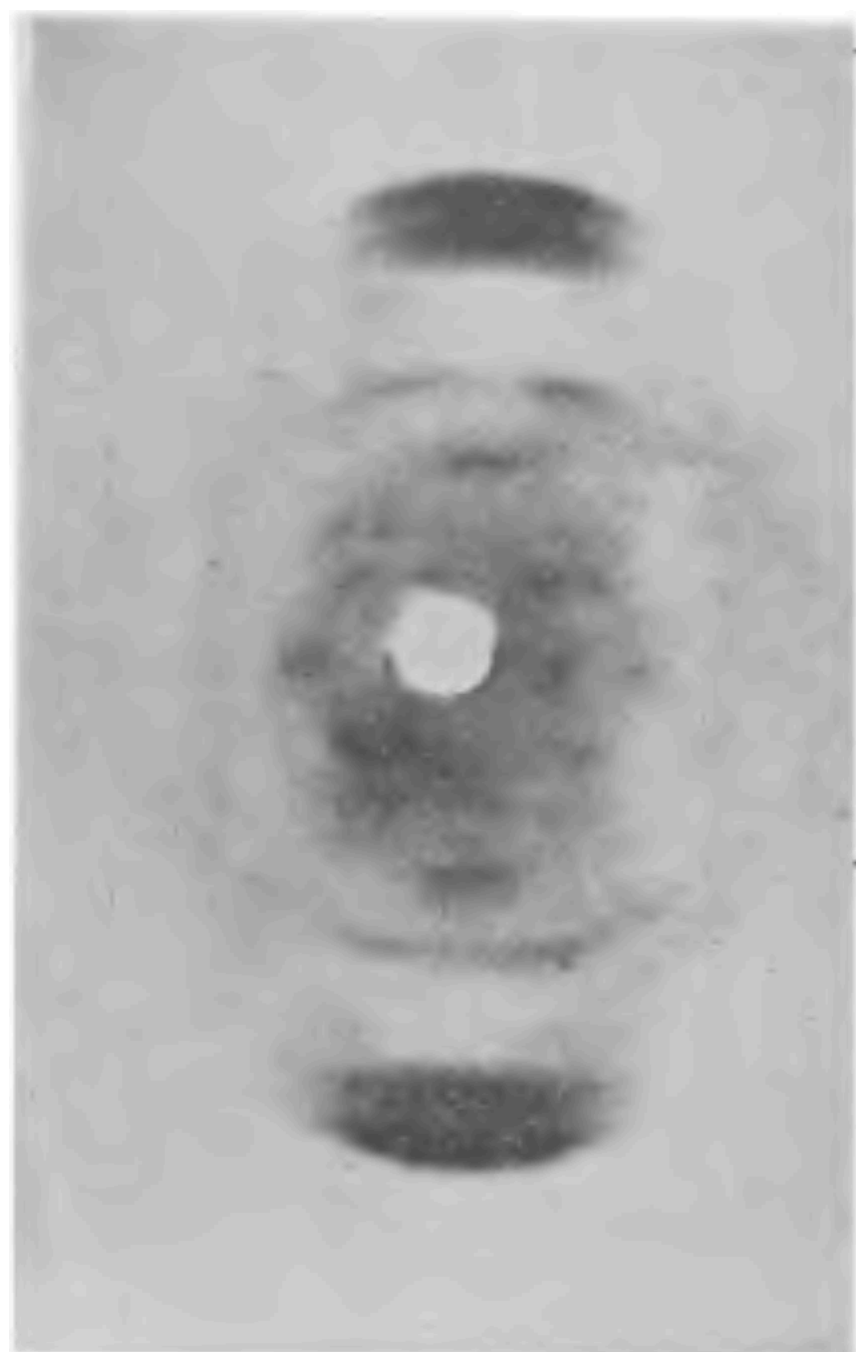


Fig. 1 *c*. Mixture of B and Z patterns obtained at 40% humidity (22°C) after performing the humidity cycle (see text).

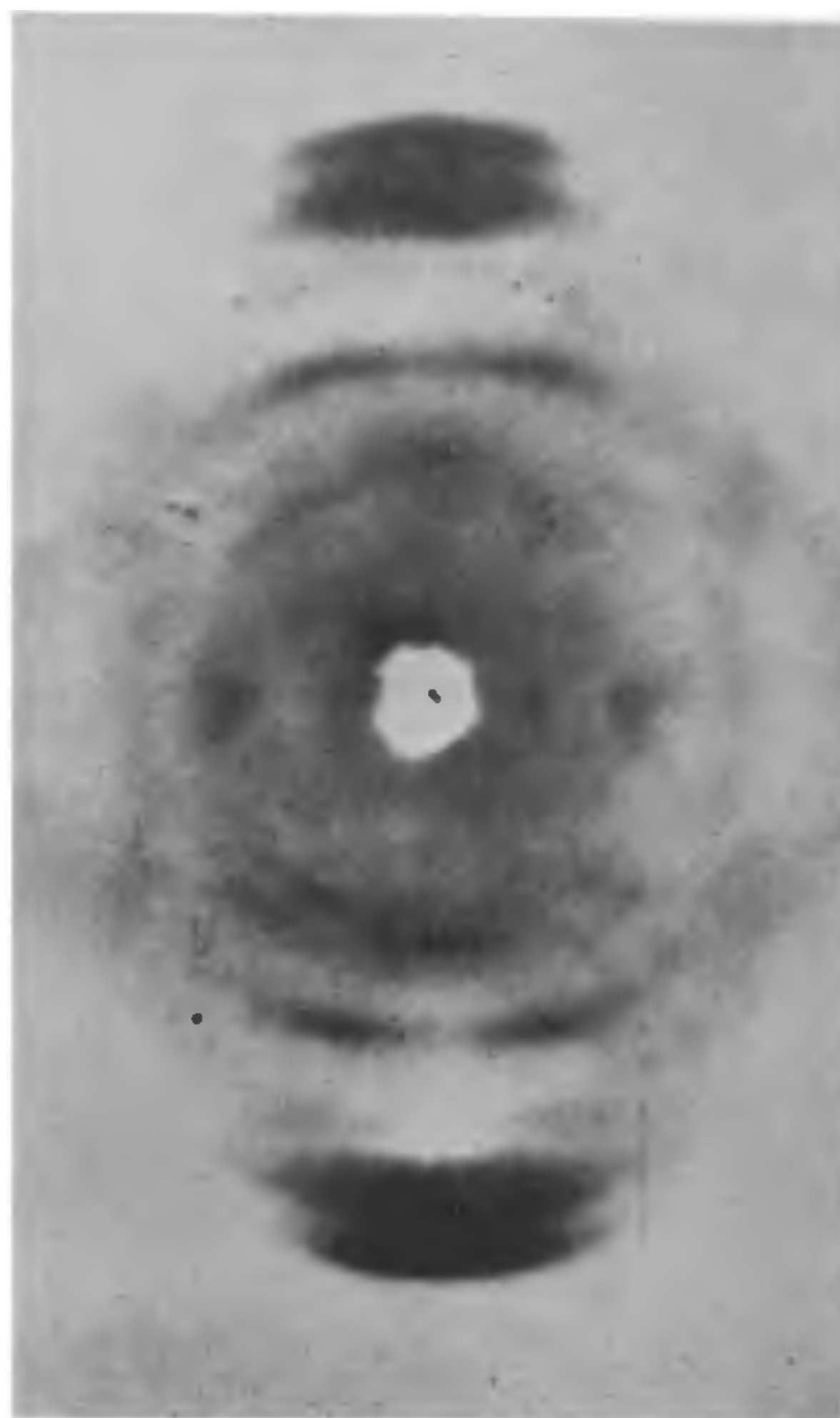


FIG.1.*d*. Characteristic Z pattern of the same fibre obtained after humidity cycle and prolonged equilibration at 40% humidity (22°C),

## CONCLUSION

It is obvious from the above results that not only is it possible to obtain with ethanol treatment a Z pattern similar to that obtained from high salt poly(dG-dC)<sup>16</sup>, but also that the same fibre can give rise to both B and Z patterns in the solid state. Since this smooth transition is observed without any drastic treatment such as prolonged heating or cooling, this clearly demonstrates that this transition from B to Z cannot be associated with a change of handedness of the duplex. The conclusion drawn by Arnott *et al*<sup>16</sup> that such a transition from B to Z in poly(dG-dC) necessarily involves a change in handedness of the duplex is therefore untenable and the overall handedness of the duplex in both forms should be the same. The conditions under which we have observed the B to Z transition do not leave any possibility for breaking and remaking of hydrogen bonds which are unavoidable if a right-handed Watson-Crick double helix in the B form were to undergo transition to a left handed Z structure; such a transition, on the other hand, can be understood on the basis of a RL model of DNA as proposed by Sasisekharan and co-workers.

## ACKNOWLEDGEMENT

This work was supported by the Department of Science and Technology, New Delhi.

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