# BRIEF COMMUNICATION

## ELECTRON DIFFRACTION FROM SINGLE CRYSTALS OF DNA

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ABSTRACT Crystals of DNA have been grown in a form suitable for study by electron diffraction and electron microscopy. Preliminary electron diffraction patterns have been obtained from the crystals, representing the first diffraction patterns of any type that have been obtained from single crystals of highly polymerized DNA. The patterns, obtained from frozen, hydrated crystals with the beam approximately parallel to the DNA strand axis, show a hexagonal geometrical arrangement with a (1,0) Bragg spacing of 23.1 Å.

Structural studies on DNA have been based almost entirely on x-ray diffraction data obtained from oriented or polycrystalline fibers of DNA. The double helical models derived from the limited x-ray data have been refined extensively using well-documented stereochemical and other data (Langridge et al., 1960; Arnott and Hukins, 1972, 1973), and are consistent with a wealth of biochemical and physical data. However, variability in the structure of DNA, depending upon salt and hydration conditions as well as base composition, has been widely recognized, and marked departures from the Watson-Crick model have been proposed from time to time. X-ray studies on single crystals of small oligonucleotides (Viswamitra et al., 1978; Drew et al., 1978) have tended to confirm the double helix model, but a structure dramatically different from the Watson-Crick model has recently been determined by x-ray diffraction analysis (Wang et al., 1979). Except for these latter studies, no direct structural determinations have been performed because of the inability to obtain diffraction from DNA single crystals.

The formation of platelet-like DNA crystals by precipitation of short DNA segments in ethanolic solution was first reported by Giannoni et al. (1969). X-ray diffraction, electron microscopy, and light microscope examination of these crystals suggest that the DNA was packed hexagonally, with the polymer axis normal to the broad face of the crystals and with the strands folded back and forth, as in crystals of other long chain polymers (Vainshtein, 1966). Further studies of crystals of this type by Lerman et al. (1976 included visualization of the cross section of the crystals by freeze-fracture electron microscopy. In this study, DNA strands were seen running through the full thickness of the crystals, verifying the chain packing as proposed by Giannoni et al. (1969) but with the absence of chain folding.

Crystals of this general type, i.e., thin platelets, would be well suited to a structural study by

electron diffraction and electron microscopy, as implemented recently in studies of some proteins (Unwin and Henderson, 1975; Henderson and Unwin, 1975). Crystals of a suitable thickness should be produced either by folding of relatively long DNA strands, as proposed by Giannoni et al. (1969), or by the use of very short strands, as in Lerman et al. (1976). We have investigated the feasibility of using such crystals for study by electron methods, and report here preliminary electron diffraction patterns obtained from single crystals.

Calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) was prepared essentially as by Lerman et al. (1976). Extensive sonication of the DNA was followed by successive ethanol fractionation of the short DNA segments. Crystals were obtained by adding sufficient ethanol to the solution to initiate precipitation of the DNA, heating to 55°–60°C, and cooling exponentially with time constants from 30 to 500 min.

A wide variety of forms of crystals has been observed in the light microscope, ranging from large ($\gtrsim$80 $\mu$m), very thin hexagons with an internal structure similar to snowflakes, to small ($\lesssim$5 $\mu$m), apparently thicker, uniform hexagons. Fig. 1 shows light micrographs of crystals formed in the third and fifth ethanol-precipitation fractions of one calf thymus DNA sonicate, illustrating the differences which have been observed between fractions. The more commonly observed morphology is that of uniformly thin hexagons.

X-ray diffraction from these crystals yielded the same results as reported by Giannoni et al. (1969) and Lerman et al. (1976), except that some orientation could be induced by centrifugation of the crystals into the capillary, and that the intense innermost ring was sometimes resolved into two rings, at 23.9 Å as previously reported, and at 26.7 Å.

The crystals in the mother liquor were found to be quite sensitive to small changes in the ethanol concentration, causing serious problems in making preparations for electron microscopy. This problem could be overcome to a large extent by replacing the ethanol with 2-ethoxyethanol (Giannoni et al., 1969). Most of the ethanol-buffer solution was removed after the crystals had been allowed to settle, and the crystals were resuspended in a solution containing ethoxyethanol and buffer. A droplet of this suspension was applied to a polylysine-treated carbon film on a microscope grid, allowed to settle, and drained briefly on filter paper

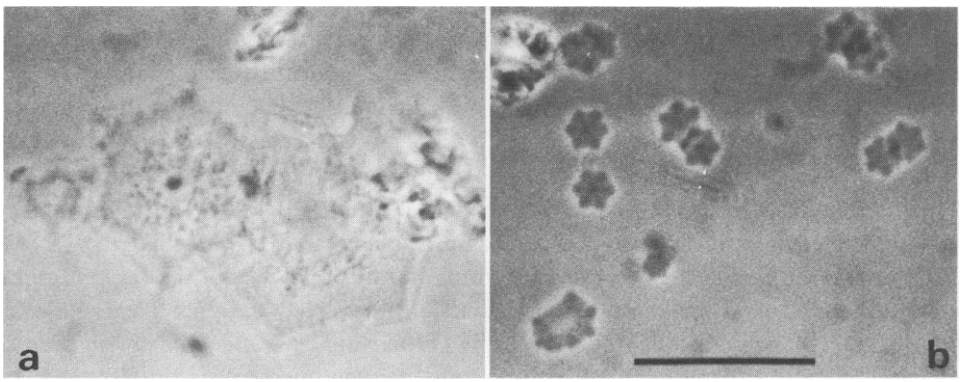


FIGURE 1 Light micrographs of DNA crystals, formed from the third (*a*) and fifth (*b*) fractions from a single calf thymus DNA sonicate. Gel electrophoresis showed that the strands in both fractions were ~200–400 base pairs long. In addition, the melting transition was somewhat broader, and occurred at ~3°C higher temperature, in the fifth fraction. Bar 50 $\mu$m.

in a chamber saturated with ethoxyethanol and buffer vapors. The grid was then quickly frozen in liquid nitrogen and transferred to the cold stage of a JEM 100B electron microscope. Rapid freezing of the thin specimens maintains the degree of hydration that is necessary for the preservation of crystalline structure in the vacuum of the microscope.

Fig. 2 is one of the first electron diffraction patterns obtained from these single crystal preparations. This pattern was obtained with the broad face approximately normal to the beam, i.e., looking down the DNA fiber axis. The hexagonal patterns, showing a 23.1-Å Bragg spacing, confirm the strand packing deduced by Giannoni et al. (1969) and Lerman et al. (1976). The difference between the spacing observed here and the 23.9Å ring in the x-ray

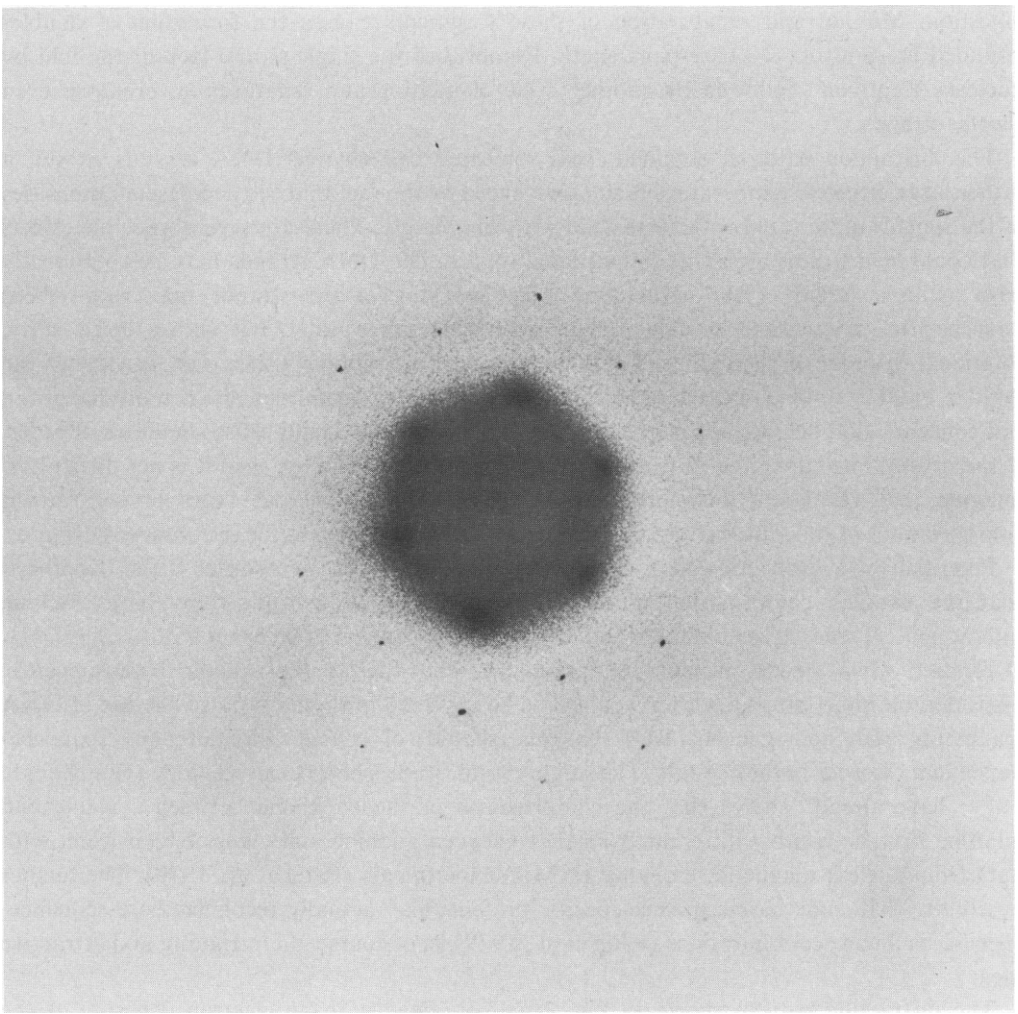

FIGURE 2 Electron diffraction pattern from a single crystal of DNA prepared as in text. The first order reflections correspond to a Bragg spacing of 23.1 Å. Diffraction spots with index (4,1) corresponding to 5.0 Å, are visible in the original negative. The print has been extensively dodged to show all the spots. The diffuse ring around the first order spots is a result of this dodging.

diffraction patterns corresponds well to the unit cell shrinkage observed by Lerman et al. in low-temperature x-ray diffraction experiments. Reflections with index (4,1) corresponding to a Bragg spacing of 5.0 Å are clearly visible in the original negative.

The thickness of the crystals has not been directly measured, but those in the preparation used for Fig. 2 were certainly too thick for structure determination by electron crystallography. Rather small tilts of the crystals were sufficient to destroy the hexagonal symmetry of the patterns, as in Fig. 2, indicating that the crystals were many unit cells thick. Work is now in progress to reduce the specimen thickness by growing crystals from DNA fragments that are as short as 35 base pairs in length. The short fragments have been produced using the synthetic DNA poly(dA-dT) · poly(dA-dT), which was first reconstituted into nucleosomes with the appropriate histones, and then trimmed to 146 base-pair segments by nuclease digestion. Melting and renaturation of these fragments causes the formation of double-stranded hairpins of ~70 base-pair length. Removal of the single-strand loop at the fold by nuclease treatment, followed by another cycle of melting and renaturation, produces even shorter strands.

The diffraction patterns obtained from sonicated calf thymus DNA crystals exhibit a rather large effective temperature factor, evidenced by the fact that the decrease of intensities of the spots is quite rapid with increasing scattering angle. There are several possible effects that could lead to this apparent crystalline disorder. The DNA strands may be rotationally free, as in a liquid crystal structure, or the packing of the strands may result from "nonspecific" interactions, which produce good long range order, but with a large, static rotational disorder of the chains. These possibilities seem unlikely, however, in view of the regular, rigid, sixfold symmetric structures of the crystals. Partial dehydration or changes in salt concentration during preparation for electron microscopy could cause some loss of order. If the crystal formation and growth involve extensive chain folding, then it is not difficult to imagine that vacancies, incomplete strand lengths, and numerous types of constrained configurations of the folded strands could occur that would account for the observed disorder.

Potentially the most interesting explanation of the short range disorder is the hypothesis that the detailed conformation of the DNA backbone is determined by base stacking interactions. If true, this could mean that the backbone conformation of the calf thymus DNA is random when viewed at high resolution, corresponding to the random base sequence. Considerable short range disorder would thus be expected in these crystals. The use of DNA fragments with homogeneous base sequence should, of course, eliminate any sequence-dependent disorder in the crystals. The single crystal studies of a hexanucleotide (Wang et al., 1979) have already shown that the conformation of the backbone between cytosine and guanine may be notably different from that between guanine and cytosine, consistent with data from nuclear magnetic resonance (NMR) experiments (Patel et al., 1979). The further possibility that some base-sequence-specific proteins may actually recognize base-sequence-specific backbone configurations (Klug et al., 1979) is, of course, an intriguing and attractive idea.

The diffraction pattern shown in Fig. 2 was obtained with an electron exposure at the specimen of $<1$ electron/Å$^2$, comparable to exposures used in recording electron diffraction patterns from some protein crystals (Glaeser and Taylor, 1978). High resolution reflections in the DNA crystals appear to have a sensitivity to radiation damage similar to that found for

protein crystals (Glaeser and Taylor, 1978) or cell membranes (Hayward and Glaeser, 1979) at low temperature.

The present experiments indicate that it is possible to prepare crystals of highly polymerized DNA in a form suitable for analysis by electron crystallography. The full structure determination can be performed as a Fourier synthesis using intensities from electron diffraction patterns and phases obtained from Fourier transforms of electron microscope images of the crystal lattice (Unwin and Henderson, 1975; Henderson and Unwin, 1975).

A direct structure determination of this type will be particularly interesting in addressing the question of DNA structure in a manner independent of x-ray techniques, and under conditions much different than those used in growing crystals for x-ray work. The axial projection of the structure will be the most straightforward to obtain, since it requires the crystal to be oriented normal to the beam, as it lies on a support film. This projection should be especially useful in view of the striking differences that can be seen between axial projections of various forms of DNA, for example the B and Z forms (Wang et al., 1979). Rodley et al. (1976) have claimed that available x-ray diffraction data from DNA fibers cannot distinguish between the double helix and a so-called "side-by-side" model, in which the strands are arranged in short helical segments of alternating handedness. Electron diffraction experiments should provide a test of this model by looking for the strong asymmetry that is predicted to appear in the axial projection of the structure of a suitably short DNA fragment.

We would like to thank Dr. S. Bram for performing the x-ray diffraction experiments, Prof. R. Stroud for making diffraction facilities available, Dr. S. Hayward for help with the frozen-hydrated specimen preparation, and Dr. R. T. Simpson for the short, synthetic DNA samples.

This work is supported by Department of Energy Contract W-7405-ENG-48.

*Received for publication 10 March 1980 and in revised form 8 August 1980.*

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