

## CRYSTALLIZATION OF DNA FROM DILUTE SOLUTION\*

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*Abstract.*—Hexagonal platelet crystals about  $1\ \mu$  across and about  $150\ \text{\AA}$  thick have been precipitated by the addition of ethanol to solutions of ultrasonically degraded, salmon sperm DNA in sodium cacodylate buffer. X-ray powder diffraction patterns from concentrated slurries of these crystals in mother liquor show intense rings at  $23.9\ \text{\AA}$  and at about  $3.4\ \text{\AA}$ ; the former arise from lateral packing and the latter from an axial periodicity of the DNA helix. Evidence is presented to indicate that native DNA helices crystallize by chain folding and that no irreversible denaturation results from the crystallization process.

Single crystals of synthetic high polymers characteristically take the form of thin platelets about  $100\ \text{\AA}$  in thickness, and up to  $10\ \mu$  or more on a side. The polymer chains are oriented with their axes normal to the planes of the platelets, and span the thin dimension of the crystals many times by folding back upon themselves at the surfaces of the broad crystal faces.<sup>1</sup> Soon after the recognition of chain folding in single crystals of polyethylene,<sup>2,3</sup> Astbury<sup>4</sup> suggested that a similar mode of crystallization might be responsible for cross- $\beta$  structures in fibrous proteins. Rich<sup>5</sup> has since proposed that the condensed state adopted by DNA molecules in certain bacterial viruses and in chromosomes might consist of chain-folded crystalline domains.

Single crystals with folded molecular chains have been grown from synthetic polypeptides<sup>6-8</sup> in both the  $\alpha$  and  $\beta$  conformations. The present investigation extends this earlier work to the crystallization of a double-stranded polynucleotide. We shall describe the morphology of lamellar crystals of DNA precipitated from water-ethanol solution. To demonstrate that in these crystals the polymer molecules are indeed folded, we must show (1) that the chain axes are oriented normal to the broad faces of the lamellae and (2) that the average length of the molecules making up the crystals is much larger than the thickness of the platelets. In addition, we must prove (3) that the molecules in the crystals retain their native helical conformations.

*Materials and Methods.*—*DNA:* Salmon sperm DNA (sodium salt) was obtained from Calbiochem, Inc. (Los Angeles). It was dissolved by gentle stirring at  $4^\circ\text{C}$  in  $0.001\ M$  sodium cacodylate, pH 6.7, to a final concentration of about  $3.5\ \text{mg/ml}$ . The ionic strength was then raised to  $0.1\ M$  by the addition of the appropriate amount of  $1\ M$  sodium cacodylate buffer, pH 6.5. The solution was subjected to ultrasonic irradiation in a 20-kc M.S.E. ultrasonic disintegrator (Model 3000, listed output 60 watts) while being cooled in an ice-water bath. 50 ml were irradiated at a time, with the larger titanium probe. The sonicated polymer was dialyzed against  $0.01\ M$  sodium cacodylate, pH 6.5.

*Preparation of crystals:* Solutions of sonicated DNA containing 2–3 mg/ml were made up to ionic strengths of  $0.05$ – $0.5\ M$  by the addition of  $1\ M$  sodium cacodylate buffer, pH 6.5. Ethanol was added, dropwise at room temperature and with stirring, to 1-ml

aliquots of the polymer solutions until the onset of turbidity. The precipitate was then dissolved by being heated rapidly to a temperature in the range 60–75°C. After being held at the final temperature for 3–5 min, the solutions were cooled slowly ( $\sim 0.3^\circ\text{C}/\text{min}$ ) to room temperature. The resulting precipitates, still immersed in mother liquor, were examined at 1250 $\times$  under a phase-contrast optical microscope. Crystals to be used for characterization were washed three times at 20°C (by sedimentation in a preparative centrifuge) to remove unprecipitated polymer. In every case, the washing solution contained water, ethanol, and cacodylate buffer in the same concentrations as originally present in the mother liquor.

*Sedimentation coefficients:* Measurements were made using a Spinco model E ultracentrifuge equipped with ultraviolet optics. The DNA samples were dissolved in 0.15 *M* sodium chloride + 0.015 *M* trisodium citrate, pH 7.0 (standard saline citrate) to a concentration of 0.02 mg/ml.

*Ultraviolet absorption:* Hypochromicity measurements were made by dissolving the polymer in 0.015 *M* sodium chloride + 0.0015 *M* trisodium citrate (pH 7.0) to a concentration of about 0.05 mg/ml, heating the solution for 10 min at 100°C in well-stoppered Pyrex tubes, and rapidly cooling it (quenching) in an ice-water bath. The optical density at 260  $m\mu$  of the unheated solution (*A*) and of the quenched solution (*A'*) were measured at 20°C and hypochromicity, defined as  $(A' - A)/A$ , was calculated. Optical density-temperature profiles were obtained with a Cary model 14 spectrophotometer equipped with a thermostated cell holder.

*Electron microscopy:* Specimens used for determination of molecular length distributions were prepared for electron microscopy by the method of Hall and Litt.<sup>9</sup> DNA both from original sonicates and from crystals was dissolved in 0.1 *M* ammonium acetate + 0.05 *M* ammonium carbonate, pH  $\sim 7$ , at a concentration of 0.02 mg/ml and was sprayed from this solution onto carbon-coated grids with an all-glass Vaponefrin nebulizer. The grids were shadowed with platinum-carbon at a distance-to-height ratio of 5:1 and lengths of deposited DNA molecules were measured from micrographs printed at a final magnification of 100,000 $\times$ .

For transmission electron microscopy, crystals were resuspended in a medium in which the ethanol content of the original mother liquor was replaced with an equal volume of 2-ethoxyethanol. This change largely prevented dissolution of the crystals caused by preferential loss of ethanol during drying. Droplets of the suspensions were placed on carbon-coated grids which, after  $\sim 2$  min, were drained with blotting paper, leaving behind a deposit of sedimented crystals. These crystals were then dried in air at room temperature and shadowed with platinum-carbon at a distance-to-height ratio of 1:1.

*X-ray diffraction:* Powder patterns from slurries of crystals in mother liquor were recorded with a Guinier-DeWolff focusing camera. Samples sealed in the specimen holder were still visibly wet with mother liquor at the end of 6–9 hr exposures.

*Results.*—Attempts to grow recognizable single crystals from undegraded salmon sperm DNA (molecular weight  $\sim 5 \times 10^6$ ) were unsuccessful. With DNA irradiated ultrasonically for times varying from five minutes to three hours, we found, as is generally true also in the crystallization of synthetic polymers, that the largest crystals could be grown from polymer of the lowest molecular weight. The shortest sonication time in which it was possible to produce identifiable lamellar crystals was 30 minutes, and the longest time used extensively was two hours. We shall restrict our discussion to crystals grown from these two sonicates.

Figure 1a is an optical micrograph showing crystals of two-hour sonicated DNA photographed while immersed in mother liquor. The crystals are hexagonal, often bilayered, platelets measuring little more than 1  $\mu$  on an edge. The largest individual crystals were prepared by adding ethanol to 0.15 per cent

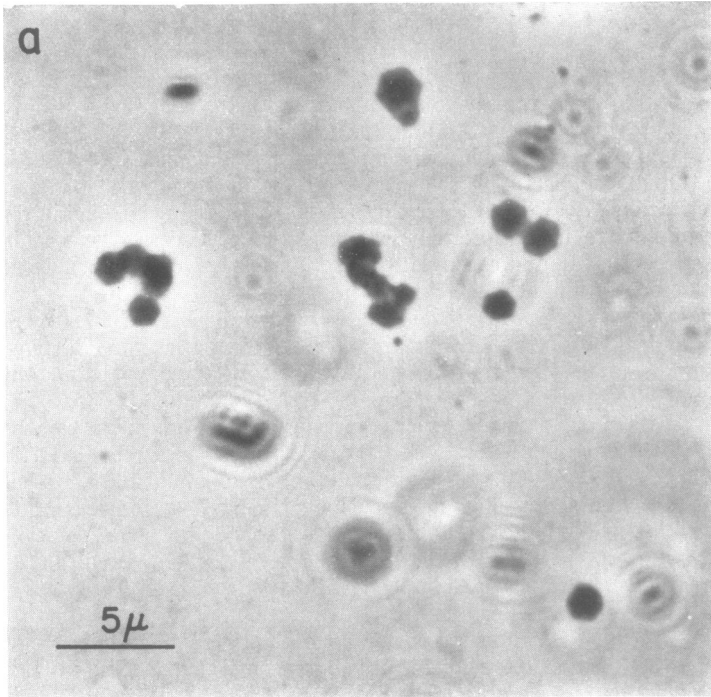


FIG. 1a.—DNA crystals prepared from 2-hr sonicate, still immersed in mother liquor. Phase-contrast optical micrograph.

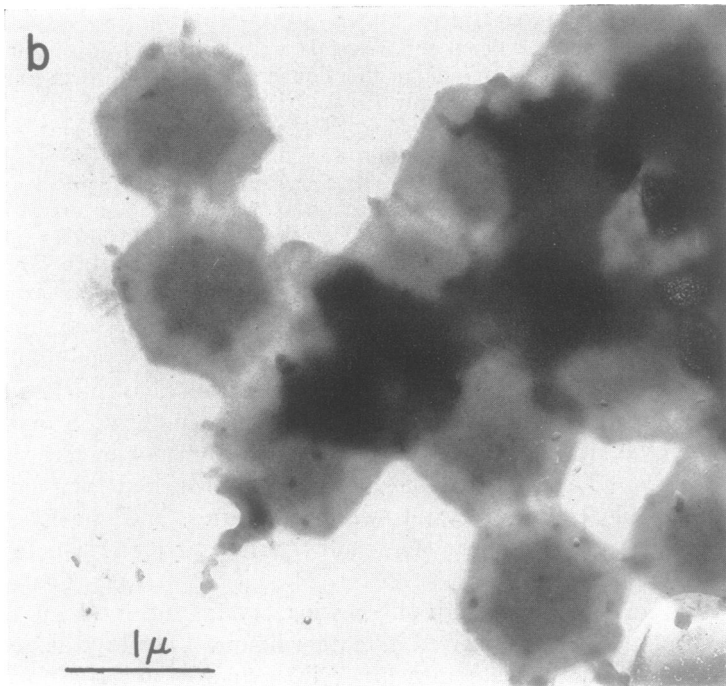


FIG. 1b.—Dried crystals as in *a*. Electron micrograph.

solutions of the DNA sonicates in 0.1 *M* sodium cacodylate, heating to 60–62°C, and cooling slowly.

There were wide differences in the influence of these various parameters on crystallite size. Ethanol content was the most critical in this respect,  $0.90 \pm 0.02$  ml (per ml of DNA solution) being required for the 30-minute sonicate,  $0.98 \pm 0.02$  ml for the two-hour sonicate, and, in general, the longer the sonication time the greater the proportion of ethanol required. The largest crystals were obtained with the two-hour sonicate from solutions heated to  $61 \pm 1^\circ\text{C}$ , while for the 30-minute sonicate temperature dependence was less sharp, the corresponding range being  $62 \pm 3^\circ\text{C}$ . On the other hand, crystal size was insensitive to changes of  $\pm 50$  per cent in ionic strength and to a tenfold reduction in polymer concentration.

DNA crystals dried after deposition onto electron microscope specimen support grids tended to collapse into shapeless masses. Figure 1*b*, an electron micrograph of crystals similar to those seen in Figure 1*a*, shows the limited extent to which their morphology can be preserved after drying in vacuum. Although the dried lamellae have a markedly less distinct appearance and tend to interpenetrate, they are still recognizable as bilayered hexagons. Crystals grown from the 30-minute sonicate were similar in shape, but generally about half as large.

To demonstrate the single-crystal character of lamellar platelets of synthetic high polymers, one resorts normally to selected-area electron diffraction, a technique that has been employed successfully with crystals of a number of synthetic polypeptides.<sup>6-8</sup> However, the loss of crystallinity in oriented DNA fibers at low relative humidities is a well-known phenomenon. It was not surprising, therefore, to find no evidence of crystalline order in vacuum-dried precipitates by electron diffraction.

On the other hand, X-ray diffraction from concentrated slurries of these hexagonal platelets yields crystalline powder patterns. Data for platelets crystallized from a 30-minute sonicate are:

$d(\text{\AA})$	23.9	15.9	13.2	11.5	3.3–3.4
Intensity	VVS	W	M	M	S(diffuse)

A pronounced tendency for the platelets to cling together in random orientations in all but the most dilute suspensions made it impractical to attempt to prepare oriented mats by sedimentation. Patterns from slurries of crystals grown under a wide range of conditions, including precipitates whose particles were too small or too irregularly aggregated to permit shapes to be identified, were essentially identical.

The thickness of the hexagonal platelets as measured by shadow casting was  $160 \pm 20 \text{\AA}$  for the two-hour sonicate and  $140 \pm 20 \text{\AA}$  for the 30-minute sonicate, each value representing an average of some 20 measurements.

Sedimentation coefficients were determined on crystals which were redissolved after first being washed to remove polymer still remaining in solution. Values of  $s_{20,w}^0$  of 4.5 for the two-hour sonicate and 8.0 for the 30-minute sonicate were obtained. From the latter value, and the relationship given by Eigner and

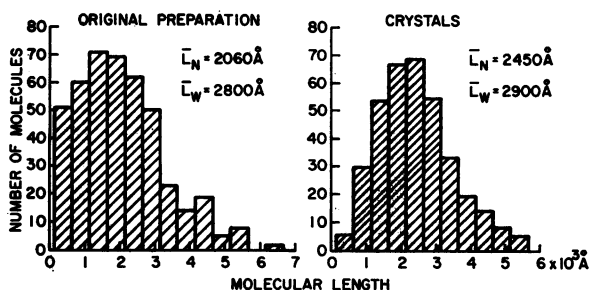


FIG. 2.—Molecular length distributions for sonicated salmon sperm DNA as determined by electron microscopy. *Left*: 30-min sonicate. *Right*: redissolved crystals of 30-min sonicate.

Doty,<sup>10</sup>  $s_{20,w}^0 = 0.116 M^{0.325}$ , a molecular weight of 460,000 can be calculated, corresponding to an average molecular length of about 2300 Å for the double-stranded helix. The value  $s_{20,w}^0 = 4.5$  for the two-hour sonicate falls well outside the range of sedimentation coefficients considered by Eigner and Doty. If their relation were even crudely applicable, it would suggest that polymer recovered from the crystals of the two-hour sonicate has a molecular weight less than 100,000 and a corresponding average molecular length less than 500 Å.

Molecular length distributions were obtained by electron microscopy only for the 30-minute sonicate. In Figure 2, distributions are shown as histograms for the original sonicate and for the washed and redissolved crystalline precipitate, representing counts of 434 and 365 individual molecules, respectively. The weight-average molecular weight calculated here for the latter is about 25 per cent higher than the value estimated from the sedimentation coefficient. Comparison of the two histograms indicates that the original sonicate has been fractionated in the crystallization process, the shorter molecules being preferentially excluded from the crystals. This conclusion is confirmed by a parallel change in the sedimentation distribution profile, as shown in Figure 3. It is apparent that some of the slower-sedimenting material has been lost in the crystallization.

In order to verify that both the ultrasonic irradiation and the crystallization procedure did not cause any denaturation of the original polymer, "melting" profiles were determined for unsonicated DNA, for the sonicates themselves, and for polymer recovered from washed crystalline precipitates. Measurements were limited to two-hour sonicates, since these would be most liable to denaturation. "Melting" profiles are shown in Figure 4 for undegraded salmon sperm DNA and for the two-hour sonicate. Their reasonably close agreement indicates that the product of ultrasonic degradation is essentially native, as was expected.<sup>11</sup> The "melting" profiles for the two-hour sonicate and the same material after crystallization are also almost identical. Hypochromicity yields values of 22 per cent for the original two-hour sonicate and 26 per cent for redissolved, washed crystals. Finally, electron microscopy of molecules deposited by spraying provides still further evidence that DNA has remained native throughout. Rodlike bodies about 25 Å in diameter are found with only occasional frayed ends parting into two strands.

*Discussion.*—We have shown that native DNA of molecular weight up to one-half million can be crystallized from dilute solution in the form of thin

platelets. (In the crystals, some hydrogen bonds between base pairs are presumably broken at the molecular folds, but this local denaturation appears to be reversible.) The hexagonal shape of the crystals together with an intense diffraction ring at 23.9 Å suggests that we are dealing with the B crystalline form of DNA<sup>12</sup> (in this case the sodium salt).

To examine the possibility of chain folding in these platelets, we must first determine helix orientation within the platelets and then compare dimensions of the crystals in the same direction with the average length of the DNA molecules. In the absence of selected-area diffraction from individual crystals, we must turn to indirect evidence for chain orientation. One such clue is provided by the diffuseness of the 3.4-Å ring in the X-ray powder patterns. This reflection arises from the purine and pyrimidine bases stacked in planes normal to the axes of the helices and would be expected to display marked particle-size broadening if helices are oriented perpendicular to the planes of the platelets. (In contrast, the 23.9-Å rings should then be sharp, as is the case.) If the crude assumption is made that it arises from particle-size broadening alone, diffuseness of the 3.4-Å ring would provide a rough measure of the thickness of the platelets. We calculate from line-width measurements on densitometer traces of the 3.4-Å ring and the Scherrer formula<sup>13</sup> that crystallite size in the direction of the helix axis lies in the range 100–200 Å. This figure agrees quite reasonably with measurements of platelet thickness by shadow casting.

Further support for the view that the DNA helices are oriented normal to the planes of the platelets is provided by analogy with the crystalline morphology of simpler helical polymers. Of the five polypeptides for which single crystals consisting of helical chains have been grown,<sup>6–8</sup> all were hexagonal platelets 200 Å or less in thickness and in all of them helices were so oriented.

Values of molecular weight of DNA from crystals of the 30-minute sonicate

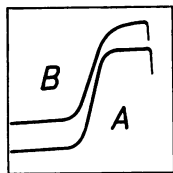


FIG. 3.—Sedimentation profiles of crystallized DNA from the 2-hr sonicate (A). Original 2-hr sonicate is shown for comparison (B). The densitometer tracings correspond to equivalent sedimentation times.

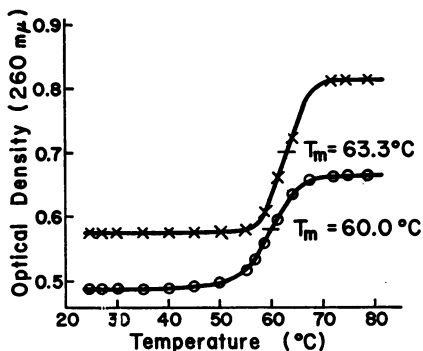


FIG. 4.—Thermal denaturation profiles of salmon sperm DNA: undegraded, molecular weight  $\sim 5 \times 10^6$ , (X), and 2-hr sonicate (O). The solvent was a mixture of equal volumes of ethanol and 0.1 M sodium cacodylate buffer, pH 6.7.

as determined by ultracentrifugation and electron microscopy are at least in rough agreement, and together indicate an average molecular length in the double-helical conformation some 15–20 times the thickness of the platelets. Within the limitations of our indirect evidence for chain orientation, we may then conclude that DNA helices do fold in the crystals.

Considered simply as polymer single crystals, the DNA lamellae are notable in a number of respects. They are unique, of course, in being composed of double-stranded molecules. One consequence—the ability to image individual, shadowed molecules in electron microscopy—provides for the first time a simple and direct means of assessing the fractionation effects common in the growth of polymer single crystals from solution. Figure 2, for example, indicates that, in the crystallization of the particular DNA sample involved, chains shorter than five to six fold periods tended to be excluded from the crystals.

From the conformational properties of native DNA there also follows the possibility that denaturation-renaturation effects may influence the crystallization process. Our results show that the largest crystals are precipitated after being heated to a temperature about midway along the melting curve, which suggests that renaturation of partly denatured helices plays a role in determining crystallite size. On the other hand, the same X-ray diffraction patterns are obtained from slurries of crystals prepared in this way as from crystals precipitated after being heated to temperatures well below the onset of the melting range. Denaturation-renaturation effects are not, therefore, critical for the crystallization process itself. The influence on crystallite size must be exerted primarily through control of nucleation density.

Potentially, the most interesting aspect of the DNA crystals is their significance with respect to biological systems. As far as is now known, the most likely instance of chain-folded crystallization of DNA in nature occurs in the bacterial viruses.<sup>5</sup> The head of the T2 bacteriophage is a polyhedral body about 1000 Å long with a hexagonal cross section about 700 Å in width. Contained within the virus head is a single, continuous DNA molecule some 60  $\mu$  in length. Packing of the DNA into chain-folded crystals analogous to the crystals we observe would be the most efficient way to satisfy the stringent requirement of fitting such a large amount of DNA into the interior volume of the head. Moreover, orientation of the polymer with the helical axes parallel to the long dimension of the head would then maintain a folded “fire-hose” configuration of the DNA strand, facilitating extrusion of the molecule through the tail of the virus into the host bacterium. Birefringence studies on T2 bacteriophage do, indeed, suggest substantial alignment of the DNA parallel to the long axis of the virus.<sup>14</sup>

North and Rich<sup>15</sup> have obtained X-ray diffraction from oriented arrays of intact T2 phage and have found that DNA-helical segments within the virus heads are packed hexagonally, with an interhelical separation varying with relative humidity up to a limiting value represented by a Bragg spacing of 23.8 Å. On the other hand, they found that fibers drawn from purified T2 phage DNA exhibit the same crystalline packing but show a continuous increase in interchain separation with relative humidity up to the point of dissolution,

and spacings of more than 30 Å were measurable. This contrast in behavior led North and Rich to speculate that the limitation on chain separation in the phage heads might be due, among other things, to the presence of chain folds. In this connection, it is interesting to note that our DNA crystals, immersed in mother liquor, exhibit a chain separation represented by a Bragg spacing of 23.9 Å.

Such comparisons with natural systems must remain speculative in view of the crystallization conditions employed in our work. Our results simply indicate that native DNA molecules of the order of 2500 Å in length can spontaneously crystallize into lamellar crystals about 150 Å thick by the mechanism of chain folding. We have made no attempt to reproduce environments even vaguely resembling those which exist within a living cell. Since we have found that conditions required for optimal growth of crystals are quite critical, it seems reasonable to expect that growth of similar crystals under "natural" conditions may require a detailed knowledge of the composition of the system involved.

*Summary.*—Ultrasonically degraded salmon sperm DNA has been crystallized from dilute ethanol-water solutions in the form of hexagonal platelets about 1 μ wide and 150 Å thick. Evidence is presented to indicate that native DNA helices crystallize by the mechanism of chain folding and that no irreversible denaturation results from the crystallization process.

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<sup>1</sup> Geil, P. H., *Polymer Single Crystals* (New York: Interscience, 1963).

<sup>2</sup> Keller, A., *Phil. Mag.*, **2**, 1171 (1957).

<sup>3</sup> Till, P. H., *J. Polymer Sci.*, **24**, 301 (1957).

<sup>4</sup> Astbury, W. T., *Discussions Faraday Soc.*, **25**, 80 (1958).

<sup>5</sup> Rich, A., *Proc. Pontifical Acad. Sci.*, **22**, 271 (1962).

<sup>6</sup> Padden, F. J., Jr., and H. D. Keith, *J. Appl. Phys.*, **36**, 2987 (1965).

<sup>7</sup> Keith, H. D., G. Giannoni, and F. J. Padden, Jr., *Biopolymers*, to be published.

<sup>8</sup> Padden, F. J., Jr., H. D. Keith, and G. Giannoni, *Biopolymers*, to be published.

<sup>9</sup> Hall, C., and M. Litt, *J. Biophys. Biochem. Cytol.*, **4**, 1 (1958).

<sup>10</sup> Eigner, J., and P. Doty, *J. Mol. Biol.*, **12**, 549 (1965).

<sup>11</sup> Doty, P., B. B. McGill, and S. A. Rice, these PROCEEDINGS, **44**, 432 (1958).

<sup>12</sup> Wilkins, M. H. F., *Science*, **140**, 941 (1963).

<sup>13</sup> Barrett, C. S., and T. B. Massalski, *The Structure of Metals* (New York: McGraw-Hill, 1966) 3rd ed., p. 155.

<sup>14</sup> Bendet, I. J., D. A. Goldstein, and M. A. Lauffer, *Nature*, **187**, 781 (1960).

<sup>15</sup> North, A. C. T., and A. Rich, *Proc. Intern. Congr. Biochem. 5th London* (Pergamon Press, 1963), p. 68.