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Cisplatin Induced Alterations In Oriented Fibers Of DNA Studied by Atomic Force Microscopy

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Oriented fibers of DNA, prepared by the wet spin method, were imaged by atomic force microscopy. It was found that an oriented fiber's substructure is an array of assemblages of DNA. For native DNA, the assemblages exhibit a characteristic width of 76 nm, a thickness of 20 nm, and appear to carry a right handed twist. Treatment with the anticancer drug cisplatin prior to wet spinning induces geometric irregularities, in the form of kinks and width distortions, into the assemblages of DNA.

Cisplatin is an anticancer drug that forms DNA adducts which inhibit the ability of various enzymes to act upon DNA (1-5). From its C D spectrum (6), it has been postulated that platination alters the secondary structure of DNA by modifying the stacking arrangements between neighboring bases. Based on N M R data (7,8), it has been postulated that platination causes kinks in the DNA chain. Computer calculation indicate that kinks of 60° may be expected (9). These calculations, however, also predict that unkinked DNA helix unwinding is an equally likely result of adduct formation. At this time, it is unresolved what conformational changes cisplatin causes in DNA, and what effect the drug has on supramolecular architecture within condensed forms of DNA.

The wet spin method is used to produce condensed fiber films of oriented DNA, where the individual fibers comprising the film arise from spinneret tubes that feed a DNA solution onto a spinning roller submerged in an ethanol-salt solution (10). DNA fiber films are used in X-ray diffraction studies, and a variety of analytical techniques have been used to examine the arrangement of DNA within

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them (11), since allowed molecular bond angles and considerations of steric hindrance are used when interpreting DNA fiber diffraction patterns (12,13). It is reported here for the first time that the oriented fibers, produced by the wet spin method, have a substructure consisting of an array of twisted assemblages of DNA, and, in addition, that treatment with the anticancer drug cisplatin induces geometric irregularities, in the form of kinks and width distortions, into the assemblages of DNA.

MATERIALS AND METHODS

The atomic force microscopes used (NanoScope II) were made by Digital Instruments, Inc., Santa Barbara CA, DNA from calf thymus was purchased from U.S. Biochemical Corp., Cleveland OH, Cisplatin (cis-diamminedichloroplatinum (II)) was purchased as Platinol from Bristol Labs., Evansville IN. The wet-spin apparatus, used to produce the oriented fibers of DNA, was constructed from available materials as described by Rupprecht (10). The roller was a 30 mm diameter delrin cylinder to which a sheet of freshly cleaved mica was adhered. The spinneret used was a Spectra/por cellulose fiber bundle purchased from Spectrum Medical Industries, Los Angeles CA.

The DNA was dissolved in a buffer of 10 mM ammonium acetate and 1 mM ethylenediamine-tetraacetic acid (EDTA) at a concentration of 2 mg/ml. Precipitation solution was 0.5 M ammonium acetate in 70% ethanol absolute. The roller speed was 50 cm/s, and the DNA solution was pumped peristaltically at a flow rate adjusted to precipitate 5 ng per mm of fiber. DNA was wound for 10 s, the roller was then extensively dialyzed in 80% ethanol absolute before being lyophilized. Cisplatin treatment was 1:1 by wt and allowed to stand on ice (with periodic mixing) for five hours before wet spinning.

RESULTS

Oriented fibers of DNA, prepared by the wet spin method, appear to be approximately 1 micron in diameter under light microscopy (Fig. 1), however, under atomic force scanning they appear to be several micra in diameter. The fibers appear to have an internal composition comprised of supramolecular assemblages of DNA (Fig. 2). These assemblages of DNA appear to undulate 10 nm vertically (SE=1,N=10 from 3 images). They have a characteristic width of 76 nm (SE=1.5,N=15 from 3 images) that is normally distributed with median at 76 nm and quartiles at 71 and 81 nm. There also appears to be a regular spacing between the assemblages (Fig. 3). At higher resolution, the assemblages of DNA appear elliptical, having a width of 76 nm, and a thickness of approximately 20 nm, in addition, they appear to carry an intrinsic right handed twist (Fig. 4).

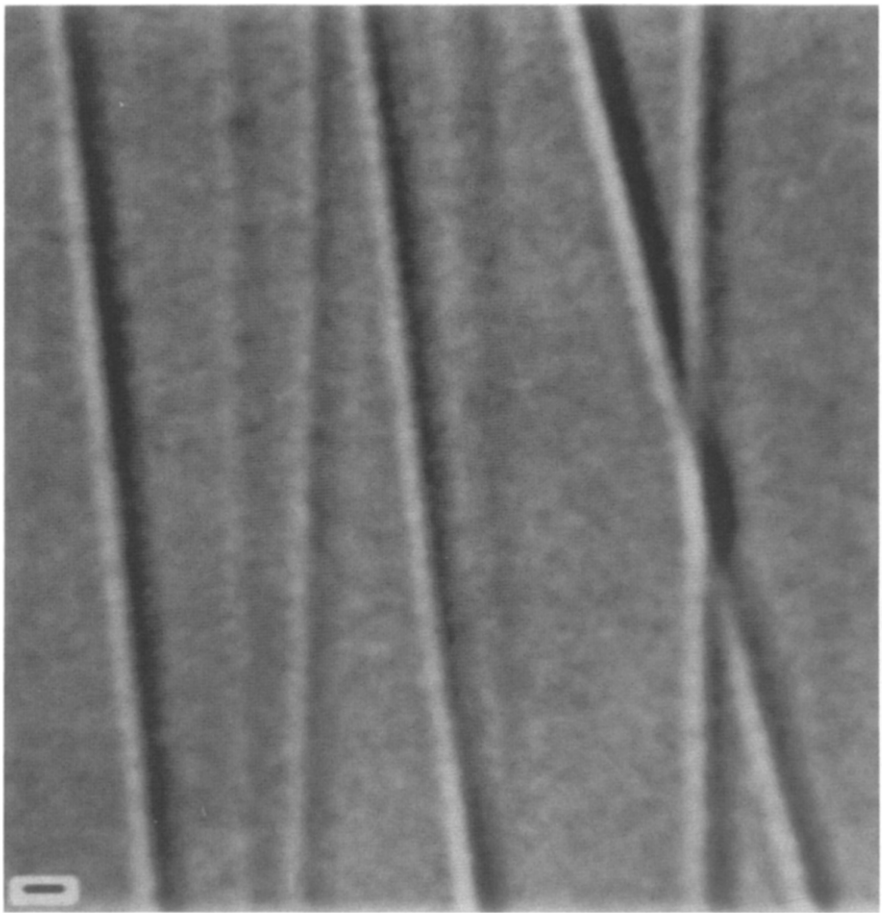

Fig. 1. Light microscopy image of oriented fibers of DNA prepared by the wet spin method. Each fiber corresponds to a single spinneret tube that feeds the DNA solution onto a spinning roller submerged in an ethanol-salt solution. Bar 1 μm.

Cisplatin induces geometrical irregularities involving kinks and width distortions into the supramolecular assemblages of DNA. The mean width of the assemblages of DNA increases to 110 nm (SE=14,N=15 from 3 images), and exhibits a distribution that is bimodal having a median at 112 nm, and quartiles at 67 and 158 nm, (Fig. 5).

DISCUSSION

The wet-spin method is designed to align individual DNA molecules by drawing them out from the spinneret tube while they are precipitated onto the surface of

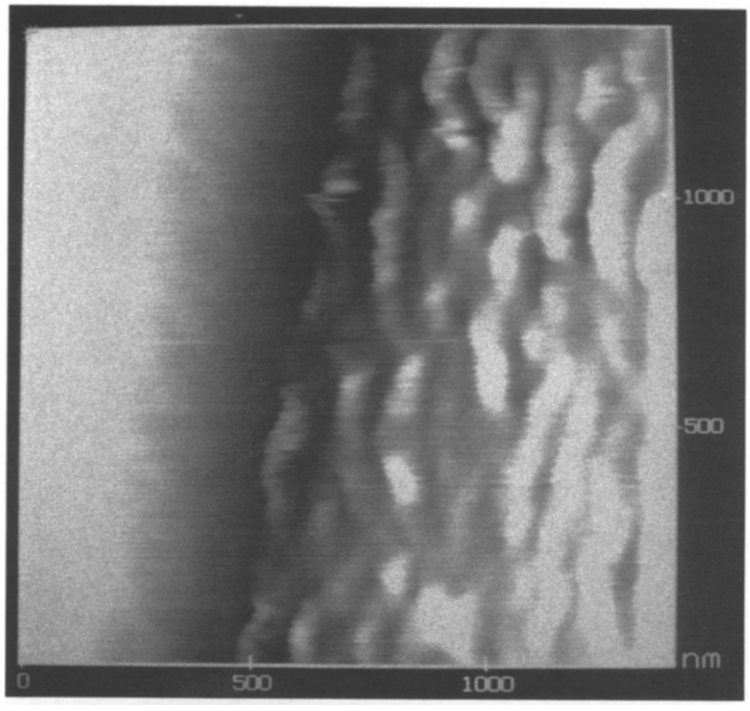

Fig. 2. Atomic force microscopy image taken at the edge of an oriented fiber.

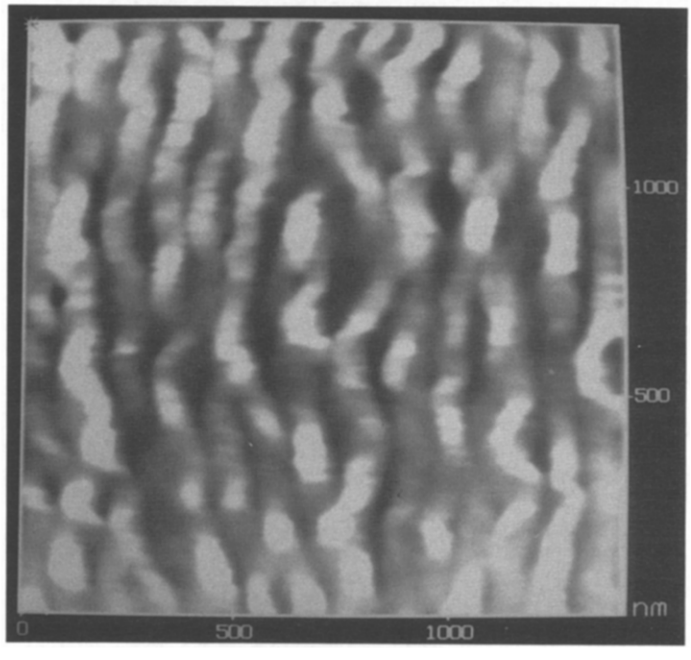

Fig. 3. Atomic force microscopy image taken near the center of an oriented fiber.

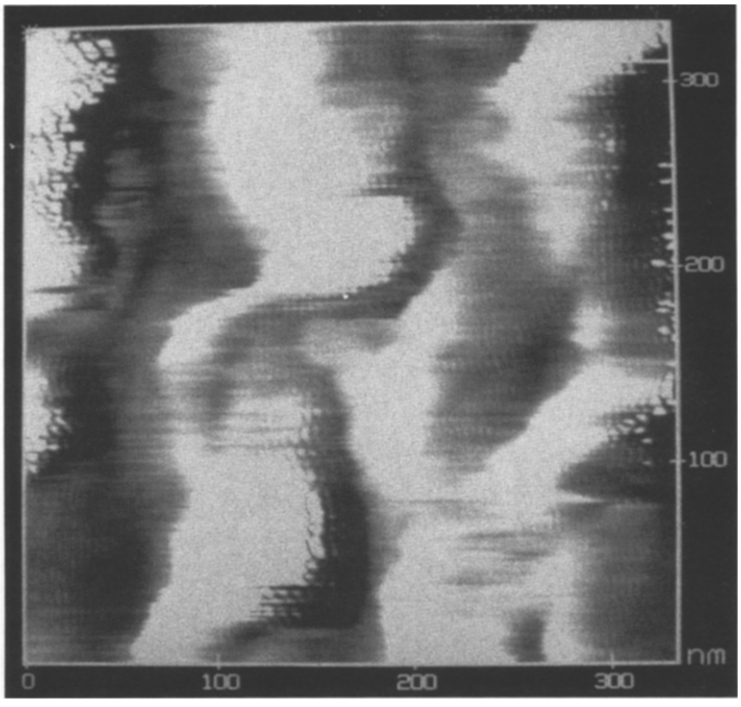

Fig. 4. Atomic force microscopy image of a single assemblage of DNA.

the roller. As two aligned DNA molecule in solution approach each other, they must share a diminishing amount of water as the ethanol and salt displace the water. The lowest energy configuration for two such helical molecules should be to contract (14) and twist about each other (15). For an added third molecule, the lowest energy configuration should be to twist about the proceeding duplex. However, a self limiting diameter of such an assembly may be reached, and would presumably be due to the phasing of the twisted DNA, governing the degree of water structuring possible between nearby molecules (16), and the increasing elastic bend energy that the outside molecules experience. At this self limiting diameter it would be energetically unfavorable for additional helical molecules to add. From this hypothetical mechanism, we may expect a regular spatial ordering into supramolecular assemblages to occur during precipitation. The supramolecular assemblages of DNA would emerge in the precipitation process and become well defined in the fully precipitated solid phase. Once such molecular ordering is in place, the molecular architecture should be insensitive

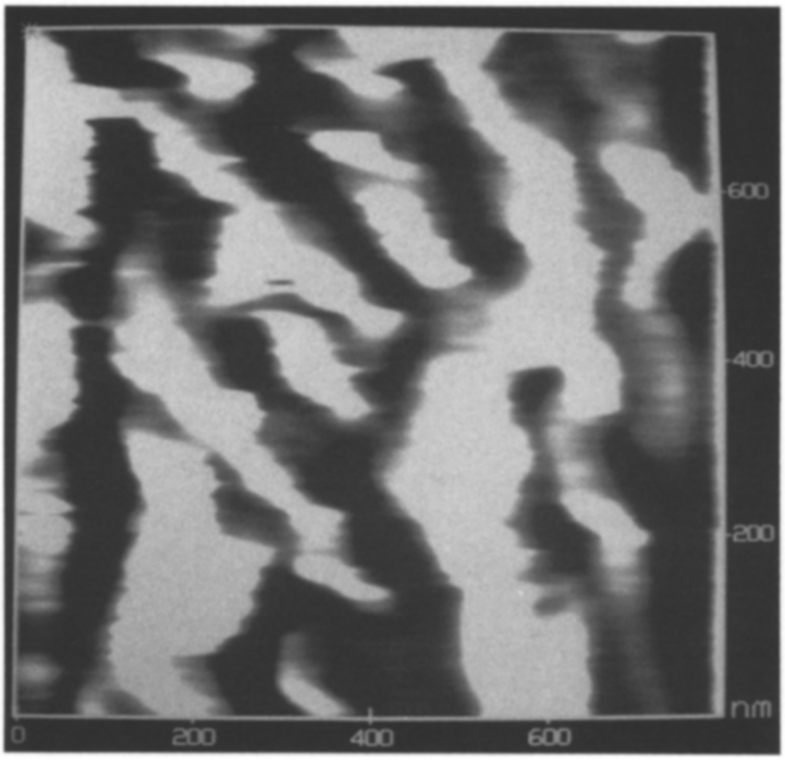

Fig. 5. Atomic force microscopy image taken near the center of an oriented fiber prepared using cisplatin treated DNA.

to wetting, and this is in fact a characteristic of DNA fiber films (17). Cisplatin adducts appear to influence the conformation of DNA sufficiently so as to destroy the regular supramolecular ordering exhibited by native DNA. This characteristic may be important in the drug's therapeutic effect since DNA is at times highly condensed in the cell.

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