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The Compaction of DNA Helices into either Continuous Supercoils or Folded-Fiber Rods and Toroids

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Summary

We have investigated by electron microscopy the mechanism of DNA compaction and have found that the double helix has the intrinsic potential to direct its own packaging into two distinctly different and mutally exclusive modes. The mode of DNA packaging is determined by the electrostatic charge density and water activity of the immediate microenvironment of the helix. The two basic structures formed by both linear and covalently closed-circular DNA are: a left-handed supercoil characteristic of minimally charge-shielded DNA, and a smooth rod characteristic of fully charge-shielded DNA. We propose that in the supercoil, the double helix is overwound (increased turn-angle), while in the rod, the helix is folded back and forth on itself. Variations of these two basic structures are the beaded fiber of DNA obtained with partially charge-shielded DNA and the toroid formed by the bending of the DNA rod and fusion of its ends in the presence of certain cations. We compare the DNA packaging inside these in vitro generated structures to DNA packaging in chromatin and viral capsids, and conclude that the packaging of DNA brought about by the use of salts and alcohol closely mimics the packaging behavior of the DNA in vivo, where it is usually complexed with histones or polyamines.

Introduction

The genetic information of most living systems is encoded in the structure of the linear polyelectrolyte DNA. These extended DNA molecules are of extreme asymmetry, while the DNA found inside viruses, bacteria and eucaryotic nuclei is highly compacted. For example, with Drosophila, the axial ratio of a purified DNA molecule is 10^6, while that of a metaphase chromosome is approximately 10. The present study is concerned with the following question. What function does the DNA helix itself have in the formation of such compacted structures?

Several lines of evidence suggest that the DNA helix has an active role in its own compaction. The basic structure of the DNA helix can be perturbed in two interrelated ways, both of which bring about changes in the hydration shell associated with the double helix. First, changes in the degree of charge neutralization of the DNA phosphates have been shown to elicit extensive changes in the hydrodynamic properties of the DNA helix (Wang, 1969; Rinehart and Hearst, 1972), while complete neutralization results in the separation of the DNA phase (precipitation) in the form of compact structures (Shapiro, Leng and Felsenfeld, 1969; Haynes, Garrett and Gratzer, 1970). Second, changes in the dielectric constant of the DNA solution have been shown to change the hydrodynamic properties of the DNA helix (Herskovits, Singer and Geiduschek, 1961; Lerman, 1971), leading to the formation of compact structures (Lerman, 1971; Lang, 1973).

This report demonstrates that although several different structures can be observed upon DNA collapse, there are only *two* fundamentally different conformations in which the DNA helix is packaged in these structures. While we have generated these structures in vitro using simple salts to regulate the degree of charge neutralization and with alcohol as the driving force for the compaction, the two basic structures obtained are very similar to those which the DNA assumes inside eucaryotic cells and inside viral capsids.

Results

Supercoiled DNA Structures

Collapse of Minimally Charge-Shielded DNA

When an uncharged carbon film mounted on an electron microscope grid is touched to a dilute solution of DNA in 1 mM Tris (pH 7.5), the majority of the DNA which remains associated with the grid is still in solution within the droplet clinging to the face of the grid. This has been demonstrated previously by showing that if the excess solution is withdrawn from the support film, only an occasional extensively *stretched* DNA molecule is observed on the grid (Eickbush and Moudrianakis, 1977). If this grid with its associated droplet of DNA solution is immersed in 95% ethanol, however, DNA can be observed on the grid as the fibrillar structures shown in Figure 1. A radical collapse (compaction) of the DNA due to the removal of water from the DNA helix has occurred, and a portion of this collapsed DNA has become associated with the support film. This alcohol-induced collapse is either uniform throughout the entire length of the DNA, giving rise to the smooth fibers seen in Figure 1a, or it exhibits small irregularities, giving rise to fibers that appear either beaded or with knob-like protrusions along their long axis (Figure 1b).

To understand the arrangement of the DNA in these collapsed fibers, we took five types of DNA molecules (viral and plasmid) ranging in molecular

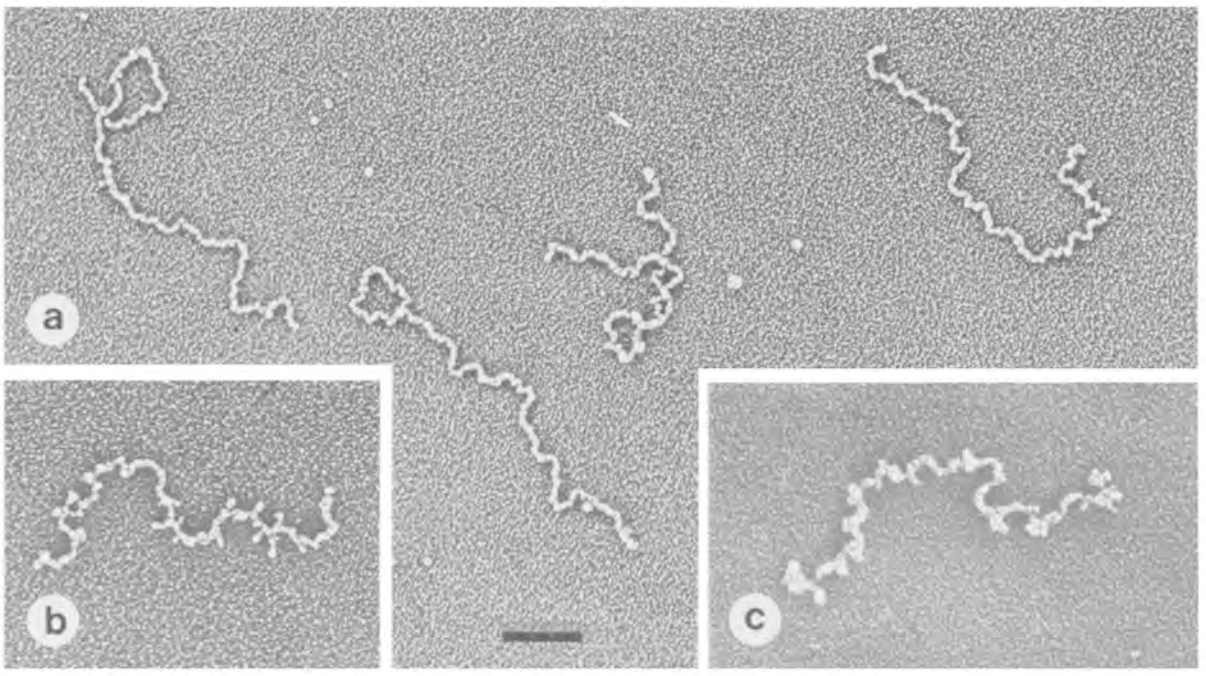

Figure 1. DNA Collapsed at Low Ionic Strength

(a) λ DNA collapsed directly onto untreated carbon-coated grids from 1 mM Tris (pH 7.5) with 95% ethanol. Uniformly collapsed fibers typically observed.

(b) λ DNA fiber generated as in (a), but with protrusions along its longitudinal axis.

(c) λ DNA collapsed in a solution of 1 mM Tris (pH 7.5) by the addition of 19 vol of 100% ethanol. The collapsed DNA was subsequently adsorbed from the ethanol solution onto glow-discharged grids. Bar = 2000 Å.

weight from 3 to 76 × 10^6 daltons (see Experimental Procedures), individually collapsed them from 1 mM Tris (pH 7.5) with ethanol, measured the dimensions of the resulting compact fibers and summarized the results in Figure 2. Since stepwise immersion of the DNA first in 70% ethanol followed by 95% ethanol completely eliminated the production of the irregular fibers, thereby giving rise to only uniformly smooth fibers, this preimmersion step was used to generate all the structures used in obtaining these measurements. As seen in Figure 2, the lengths of the compact fibers generated in the alcohol washes are directly proportional to the DNA molecular weights, while the cross-sectional area of the fibers remains invariant—that is, it is independent of the DNA molecular weight. In other words, DNA molecules of increasing molecular weights have all undergone an 8.6 fold linear compaction yielding cylindrical fibers of proportional length but with a constant outer diameter of 80–90 Å.

The independence of the diameter of the collapsed fibers from DNA length indicates that the DNA helices in these structures are folded, or more probably coiled, perpendicular to the long axis of the fibers. Direct evidence for this type of coiling is obtained from close observation of collapsed fibers that have been partially stretched during their attachment to the carbon film (arrows in Figure 3). Such stretched regions clearly reveal the left-hand supercoiled state of the DNA inside the fiber.

We next sought to determine the mechanism of the supercoiling of the DNA double helix that is responsible for the formation of these cylindrical fibers. This type of supercoiling would be produced if the fiber were generated by a simple continuous wrapping of the B form DNA helix about a hypothetical axis down the cylinder-axis of the fiber. While linear or open-circular DNA could easily accommodate this wrapping, covalently closed-circular DNA molecules would have difficulty forming fibers compacted in this manner since their ends are not free to rotate to relieve the torque generated by the winding helix. When closed-circular pDmre60 DNA was collapsed with 95% ethanol, smooth circular fibers were produced (Figure 4), indicating that such a simple wrapping model for the formation of the collapsed fibers is not correct. Instead, we propose that the alcohol-induced dehydration of the DNA changes the turn-angle (screw-angle) between consecutive base pairs in the DNA helix, and this in turn generates the supercoil. This point is elaborated upon in the Discussion.

It should be noted that fibers generated by

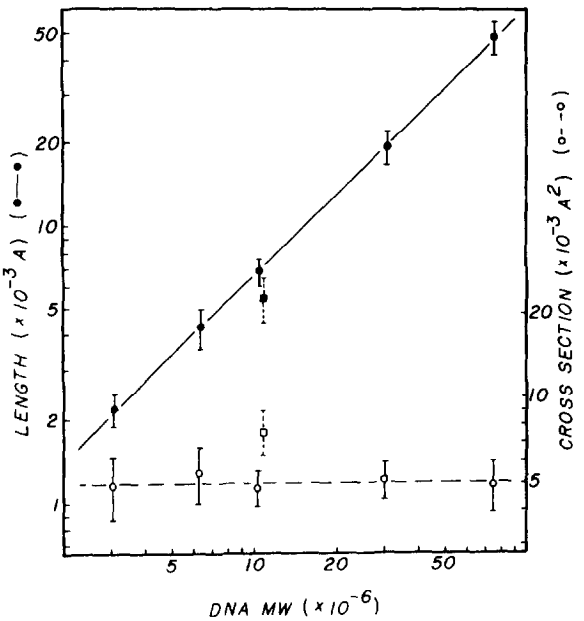

Figure 2. Dimensions of the Low Ionic Strength DNA Collapsed Fibers as a Function of DNA Molecular Weight

DNA in 1 mM Tris (pH 7.5) collapsed onto carbon films with a 70% ethanol followed by a 95% ethanol immersion. Closed symbols represent the average fiber length measured from platinum-shadowed preparations. Open symbols represent the average cross-sectional area of the fibers, *calculated from the diameter of negatively stained* fibers assuming the fibers to be uniformly cylindrical. Error bars indicate the standard deviation of the measurements. The following DNA samples were used: SV40, 3.1 × 10^6 daltons; PM2, 6.4 × 10^6 daltons; pDmre60, 10.6 × 10^6 daltons; λ, 30.8 × 10^6 daltons; and T5, 76 × 10^6 daltons. Circles represent form II or form III DNA molecules, while squares represent covalently closed form I (supercoiled) DNA.

closed-circular DNA molecules are both shorter and wider than fibers produced from linear DNA (closed squares in Figure 2). Whether this behavior is indeed a result of a small amount of torque being generated in the helix or is due to the supercoils already present in the closed-circular molecule cannot be determined at this time.

To what extent does the carbon support film contribute to the formation of the structures described here? In this study, we have purposely used untreated carbon support films to minimize film surface-DNA interactions. Thus while the number of DNA molecules adsorbed to the grid was sometimes low, the structures reported here were all well defined and characteristic of each of the salt-alcohol combinations used. If carbon-coated grids made hydrophilic by the glow discharge treatment were used, the better adsorption of the DNA to the support film resulted in either fully extended (uncollapsed) or partially collapsed structures. As a more rigorous *direct* test for "grid artifacts," we also formed the compacted structures of DNA "in solution" and then deposited them on the grid. For this, 19 vol of 100% ethanol were added to a tube containing a *dilute* solution of DNA (5 μg/ml) in 1 mM Tris (pH 7.5), the system was mixed, and a small droplet of the new solution was then applied directly to the carbon film and allowed to air-dry without further treatment. As shown in Figure 1c, this procedure yields fibers similar to those shown in Figure 1b. We thus conclude that the compacted state of DNA seen in Figure 1 represents the true state of DNA compaction and is not the result of "grid-induced artifacts."

It is interesting to note that Herskovits et al. (1961) found that in the absence of salt, DNA can exist in 100% ethanol as a stable, molecularly disperse solution. The major response of the DNA to the alcohol was some form of internal compaction, since the sedimentation velocity of the DNA increased while the viscosity of the solution decreased. Our results indicate that this DNA is collapsed in the form of a coiled fiber. Herskovits et al. (1961) also found that if methanol is substituted for the ethanol, a less radical compaction of the DNA occurs. Consistent with this is our finding that λ DNA collapsed onto carbon films with 95% methanol results in smooth supercoiled fibers with a length of 3.09 ± 0.13 μM and a diameter of 49 ± 8 Å. Linear compaction of the DNA with methanol is thus 5.4 fold, or only 60% as great as the ethanol-induced collapse.

The compaction of DNA by alcohol into uniformly smooth fibers has been reported previously by Lang (1973), who found that when T7 DNA in low ionic strength is immersed in 10% ethanol, the DNA collapses into smooth fibers with a 4.2 fold linear compaction. In that study, Lang also reported that when the concentration of ethanol was increased to 95%, short rod-like structures were formed in which the DNA was compacted 80 fold, and he attributed these structures to higher orders of DNA supercoiling. As is discussed in more detail below, however, these rod-like structures do not result from the DNA assuming a supercoiled configuration. Instead, the formation of these DNA rods is a direct result of the fact that, in addition to increasing the ethanol concentration of the collapsing solution to 95%, Lang simultaneously increased the salt concentration of the DNA solution to 150 mM ammonium acetate, a concentration which we find brings about a qualitatively new form of DNA packaging, the folded fiber.

The Collapse of Partially Charge-Neutralized DNA

The smooth supercoiled fibers described in the previous section are extremely sensitive to the ionic strength of the DNA solution. Ammonium acetate was used in most of these studies because both its ions are volatile, and this removes the problem of salt crystal formation on the surface of the dried carbon film. Thus, while the results with ammonium acetate are emphasized, qualitatively

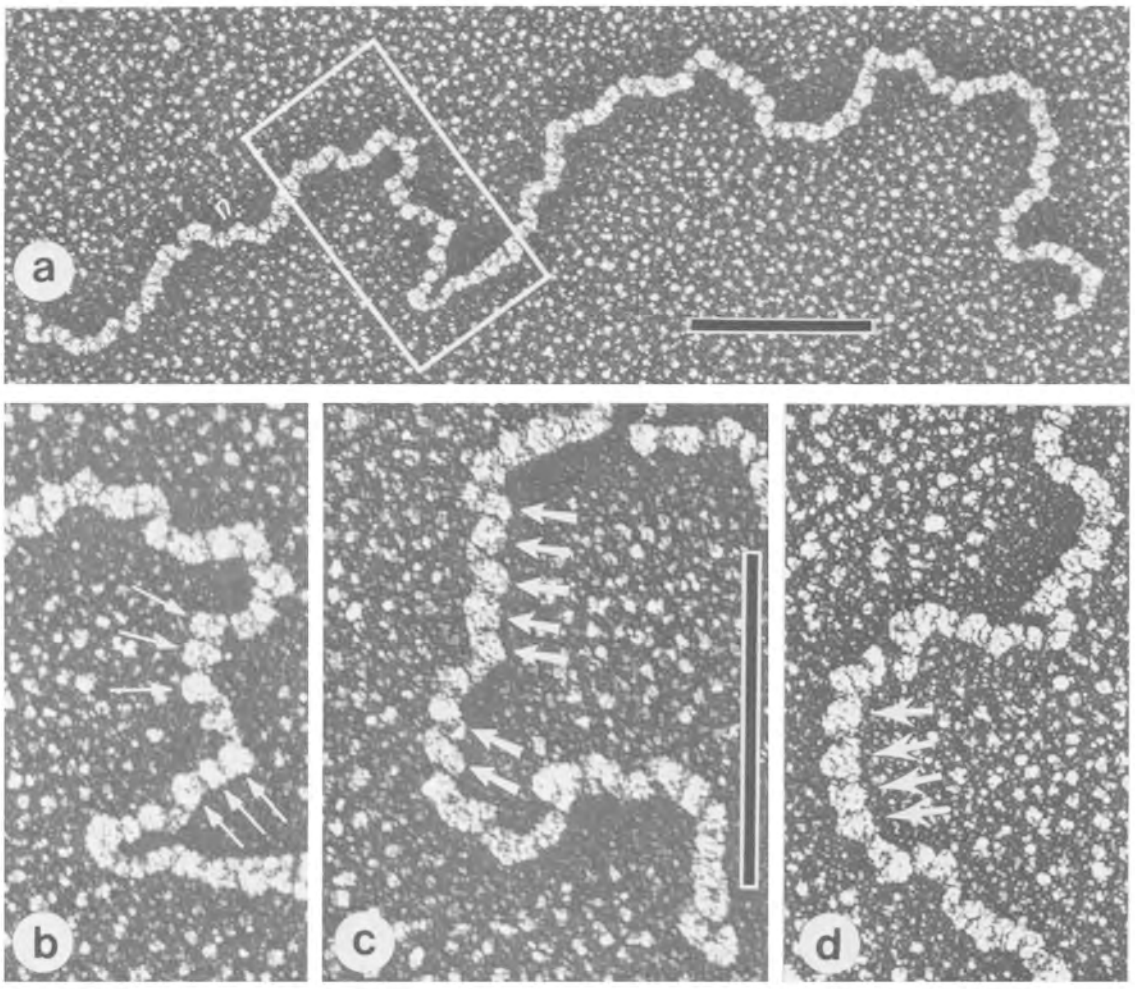

Figure 3. Coiled Nature of the Fibers of DNA Collapsed from Low Ionic Strength

Phage λ DNA collapsed onto carbon films as in Figure 1, but examined at higher resolution to reveal the coiled arrangement of the DNA (see arrows). (a) Low magnification view of a typical fiber; (b) higher resolution view of the rectangle in (a); (c and d) higher resolution views of segments from two other DNA fibers. The left-hand nature of the homonemic supercoil can be ascertained from (c). Bars = 2000 Å.

similar results were obtained with other monovalent or divalent cations. As one gradually increases the salt concentration, the DNA fibers generated by 95% ethanol collapse become more irregular, but clearly decrease in length and increase slightly in diameter. DNA fibers collapsed from a solution of 3 mM ammonium acetate are already significantly shorter compared to the fibers of Figure 1. By 10 mM, and extending as far as 50 mM ammonium acetate, a reasonably uniform maximal compaction of the DNA into fibers occurs. Figure 5 shows DNA molecules in 10 mM ammonium acetate and collapsed by 95% ethanol. These fibers are approximately 0.5 μM in length, which would correspond to a 30–35 fold linear compaction of the DNA. While measurements as extensive as those in Figure 2 have not been conducted, routine observations of the structures formed with DNA of variable molecular weights indicate that the diameter of these intermediate ionic strength fibers remains constant while their length varies. In other words, the DNA is coiled (packed) perpendicular to the long axis of the fiber. Unlike the smooth, low ionic strength fibers shown in Figure 1, however, these intermediate ionic strength fibers appear to be composed of individual subunits of compacted DNA rather than of uniformly coiled DNA. This subunit (or beaded) appearance of the fiber is most clearly revealed in negatively stained preparations (Figure 5b) or in shadowed preparations where the DNA has been partially streaked (Figures 5c–5f). These beads, whose size varies somewhat with the type or precise amount of salt used, are approximately 150 Å in a diameter.

While it is clear that partially charge-shielded DNA collapses into a conformation generally simi-

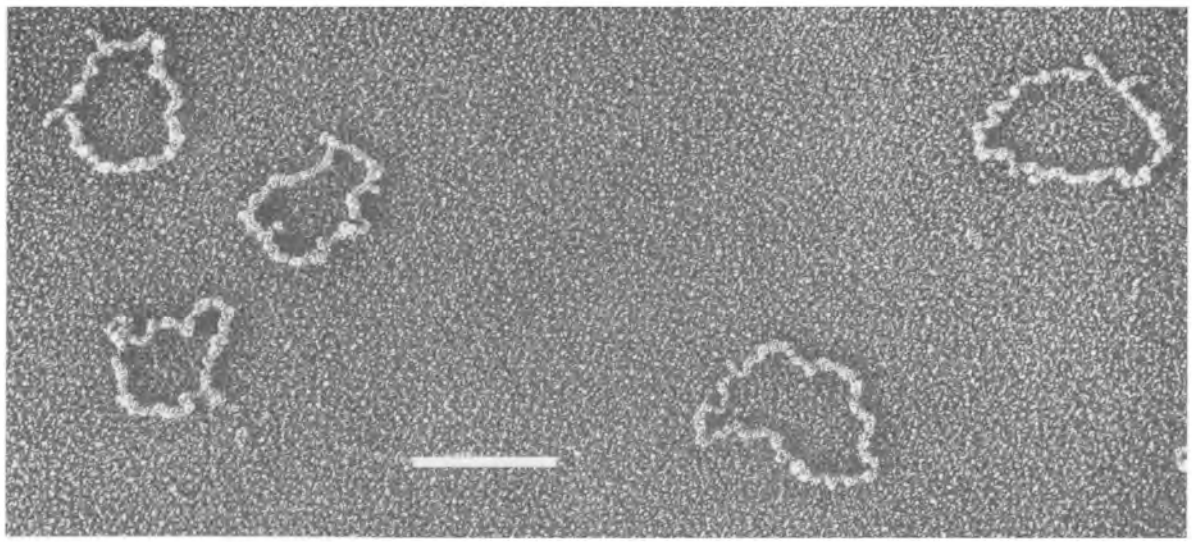

Figure 4. Closed-Circular pDmre60 DNA Collapsed at Low Ionic Strength
Bar = 2000 Å.

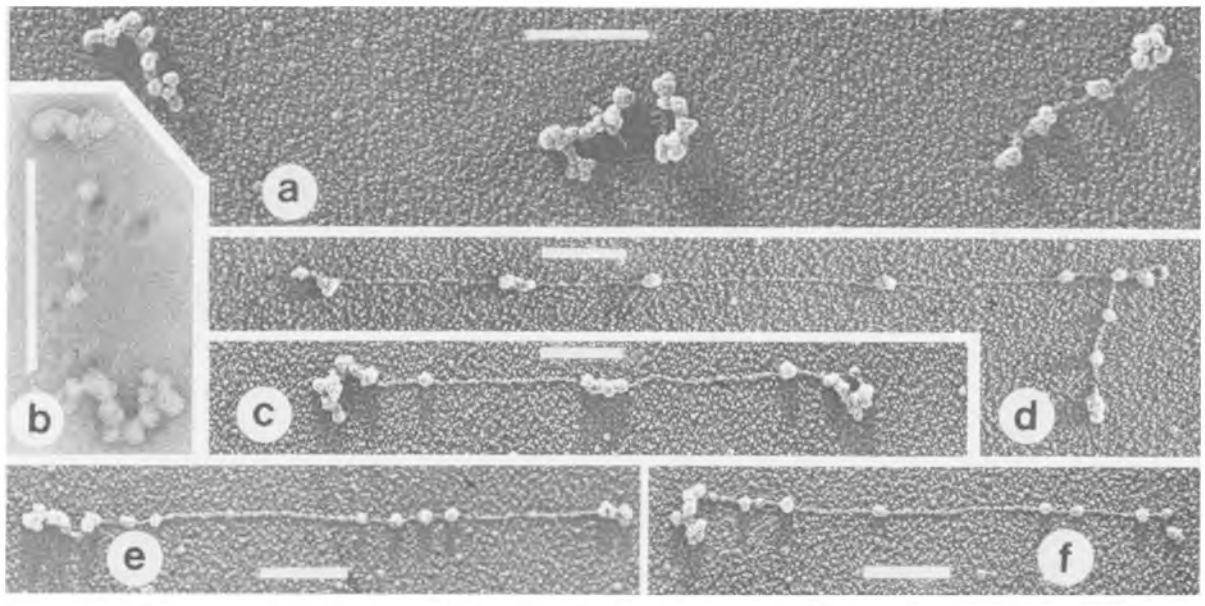

Figure 5. DNA Collapsed at Intermediate Ionic Strength
λ DNA collapsed directly onto carbon-coated grids from 10 mM ammonium acetate, 1 mM Tris (pH 7.5) with 95% ethanol. (a) Unstretched beaded fibers typically observed; (b) negatively stained beaded fiber; (c-f) examples of beaded fibers that have been *stretched* during their adsorption to the carbon film. Bars = 2000 Å.

lar to that of the coiled fibers described in the previous section, the precise path taken by the DNA inside the beads remains unclear, as is the mechanism of their formation. For now, we wish to emphasize that the contribution of the carbon film to the generation of these beaded DNA structures is minimal (if there is any at all), as demonstrated by our ability to form the compact structures "in solution" prior to adsorbing the DNA onto the film. A solution of λ DNA (5 μg/ml) in 10 mM ammonium acetate was mixed in a test tube within 19 vol of 100% ethanol, and a droplet of the mixture was deposited onto a grid and allowed to air-dry without further treatment. The structures observed had the same beaded appearance as those formed by collapsing the DNA directly onto the carbon film.

The finding that naked DNA can be compacted into a beaded structure which by morphological criteria alone is virtually indistinguishable from

that of the nucleosome necessitates the exercise of great caution in the interpretation of electron micrographs of DNA or chromatin preparations treated with dehydrating solvents.

Folded-Fiber DNA Structures

Collapse of Completely Charge-Shielded DNA into Rods

As one increases the salt concentration of the DNA solution beyond that required to generate beaded fibers—that is, 10–50 mM ammonium acetate, the DNA structures formed by immersion in 95% ethanol become ill defined. Gradually, however, and by 0.15 M ammonium acetate, a new type of collapsed structure emerges which can be best described as rod-like (Figure 6a). By analogy to the fibers described in the previous two sections, one might predict that these rods are formed through compaction (coiling) of the DNA perpendicular to the long axis of the structures. The results shown in Figures 6b–6d and 7, however, clearly demonstrate that this is not the case. Rods made by the collapse of DNA molecules of increasing molecular weight are all approximately 2000 Å in length, but now their cross-sectional area is proportional to the DNA molecular weight. This indicates that DNA compaction into these rods is accomplished by a *folding* of the DNA parallel to the long axis of the structures. In other words, the DNA helices are packaged in parallel arrays approximately 2000 Å in length, with the DNA folding back and forth on itself (a 180° bending) at the ends of the rod. In the case of the shortest DNA tested (SV40), there is enough DNA for only nine folds, while with the longest DNA tested (T5), over 170 such folds can be accommodated.

If the DNA helices in these rods are indeed in the folded-fiber arrangement, a simple 180° folding of the DNA helix at each end of the rod would not generate torque in the DNA helix, and one could predict that closed-circular DNA would give rise to *normal* rods. Consistent with this hypothesis, covalently closed-circular SV40 and pDmre60 DNA both give rise to rods (squares in Figure 7) *identical* to their linear DNA counterparts.

While 0.5 M ammonium acetate was used for the measurements in Figure 7, 0.5 M NaCl, 0.5 M CsCl and 0.003 M $MgCl_2$ have also been tested. With PM2 DNA, all cations tested gave rise to rods 1800–2000 Å in length and 105–120 Å in diameter.

It has long been known (Geiduschek and Gray, 1956) that the addition of ethanol to a salt solution of DNA to a final concentration of >65% alcohol results in the precipitation of the DNA due to the separation of a DNA phase from the solvent. The rods in Figure 6 demonstrate the result of the separation of the charge-neutralized DNA phase from 95% ethanol: each molecule is folded into a distinct "microcrystal." The folded-fiber nature of these structures is similar to the folded molecular structures widely found to occur in the separation of phases (crystallization) of organic polymers from dilute solutions (Keller, 1968). As in the case of the low and intermediate ionic strength structures, these DNA rods can be preformed in the test tube by mixing charge-neutralized DNA with 19 vol of 100% ethanol and subsequently depositing it on the grid. Solutions of DNA exposed to this high ionic strength must be maintained at extremely low concentrations (<0.1 μg/ml), since the collapsed rods readily aggregate with one another.

The collapse of DNA at high concentrations of

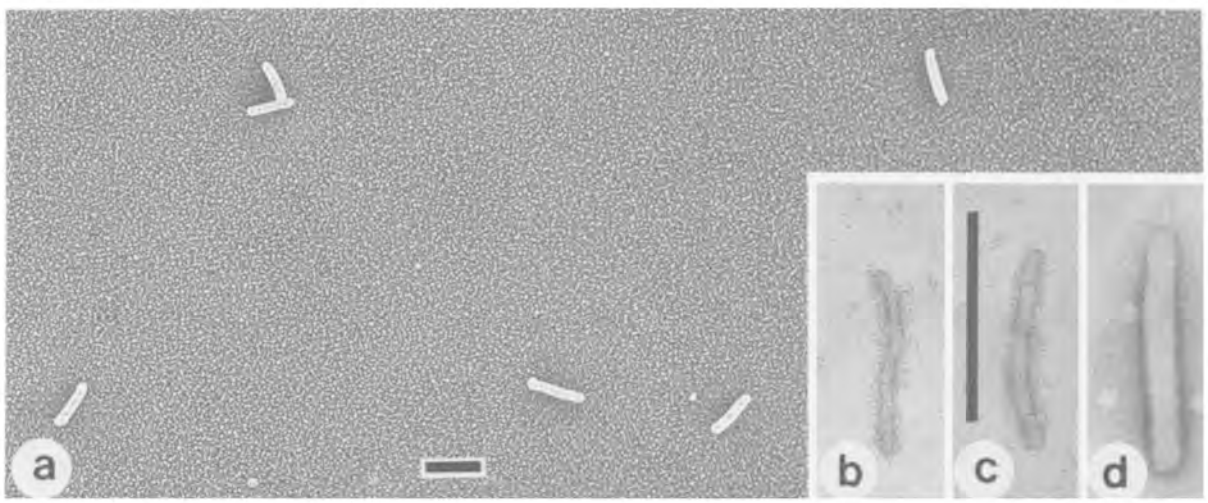

Figure 6. DNA Collapsed into Folded-Fiber Rods

DNA in 0.5 M ammonium acetate, 1 mM Tris (pH 7.5) collapsed by 95% ethanol onto carbon-coated grids. (a) λ DNA; (b) SV40 DNA; (c) pDmre60 DNA; (d) T5 DNA. Bars = 2000 Å.

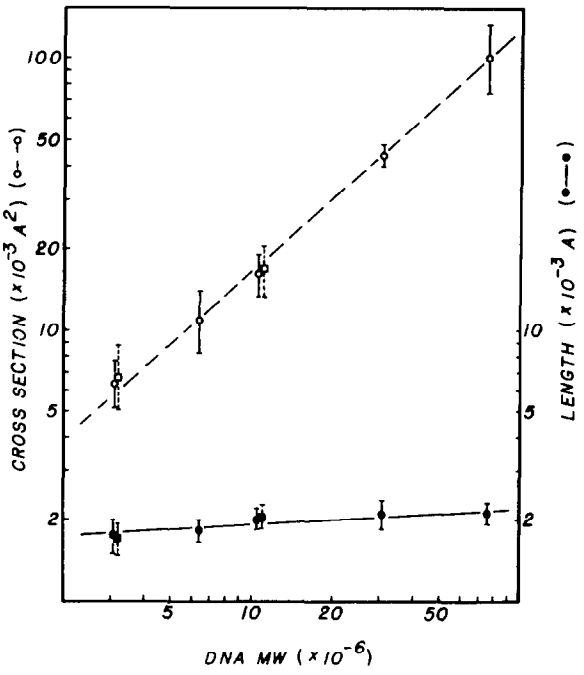

Figure 7. Dimensions of Golded-Fiber Rods as a Function of DNA Molecular Weight

DNA in 0.5 M ammonium acetate, 1 mM Tris (pH 7.5) collapsed onto carbon films with 95% ethanol. The DNA samples were identical to those used in Figure 2. Solid symbols represent the average rod length *measured from negatively stained preparations*. Open symbols represent the average cross-sectional area of the fibers, calculated from the diameters of the rods assuming them to be uniformly cylindrical. Error bars indicate the standard deviation of the measurements. Circles represent form II or form III DNA molecules, while squares represent covalently closed form I (supercoiled) DNA.

ammonium acetate into rod-shaped structures by 95% ethanol has been reported previously by Lang (1973); his interpretation of these structures, however, is entirely different from our own. Lang suggested that rods are generated by a tertiary supercoiling of the DNA (a coiled coil). In this conformation, the length of the rod would be expected to be proportional to the DNA molecular weight. This is exactly opposite the results shown here in Figure 7, where it is clearly seen that with DNA molecules of increasing molecular weight, the cross-sectional area of the rod is proportional to the DNA molecular weight, while the rod length remains constant. In a more recent report, Lang et al. (1976) concluded that circular DNA, whether open or covalently closed, is under some "topological constraint" which inhibits the formation of rods with characteristics *identical* to those formed by linear DNA. This finding is also inconsistent with the results shown here in Figure 7. We find that both closed-circular SV40 and pDmre60 DNA give rise to rods whose dimensions are indistinguishable from their linear DNA counterparts.

Lerman et al. (1976) have recently presented a freeze-fracture-etching study of crystals grown by the addition of ethanol to a solution of low molecular weight DNA in 0.5 M sodium acetate. They observed that there was a selective precipitation out of solution of DNA molecules whose lengths closely corresponded to the thickness of these crystals—that is, 2000 Å. It would appear, then, that the 180° folding of the DNA helix at the ends of the DNA rods reported here does not prevent the DNA helix from assuming a crystalline form similar to that observed with much shorter, unfolded DNA fragments.

Polylysine and the naturally occurring polyamine spermidine have been reported to collapse DNA into rod-shaped or toroidal structures (Haynes et al., 1970; Olins and Olins, 1971; Laemmli, 1975; Gosule and Schellman, 1976). In the present study, we measured the dimensions of rods generated by the collapse of DNA with polycations in the absence of alcohol and compared them to those of rods of DNA collapsed by ethanol alone. Polylysine-collapsed PM2 DNA rods (length, 2223 ± 268 Å; width, 217 ± 44 Å) and spermidine-collapsed PM2 DNA rods (length, 2322 ± 407 Å; width, 214 ± 30 Å) are nearly the same length as, but significantly wider than, alcohol-collapsed PM2 DNA rods. Comparison of metal-shadowed preparations of alcohol-induced DNA rods to those of polycation-induced rods reveals that the latter have a lower relief above the surface of the support film. If, however, before their final drying, the grids carrying polylysine-collapsed DNA are immersed in 95% ethanol, the rods became significantly more cylindrical in shadowed preparations and have a smaller diameter in negatively stained preparations. For example, spermidine-collapsed PM2 DNA rods immersed in ethanol remain 2259 ± 442 Å in length but are only 160 ± 41 Å in diameter. This suggests that the alcohol immersion has resulted in a tighter packaging of the DNA helices in the collapsed rods.

Folded-Fiber Toroids

Uniform rods are not the only structures observed when charge-shielded DNA is collapsed by alcohol or polycations. As shown in Figures 8a and 8b, toroidal or doughnut-shaped structures are frequently seen. The relative abundance of rod- and toroid-shaped structures depends upon the cation used to shield the DNA charge. For example, the alcohol collapse of ammonium-acetate-neutralized PM2 DNA yields a relative frequency of rods to toroids of 10.4, while the same ratio for $MgCl_2$-neutralized DNA is 0.75. Polylysine-collapsed DNA in the absence of alcohol has a rod-to-toroid ratio of 0.90, while spermidine-collapsed DNA yields a rod-to-toroid ratio of 0.25. It should be mentioned, however, that with the polycation-collapsed DNA,

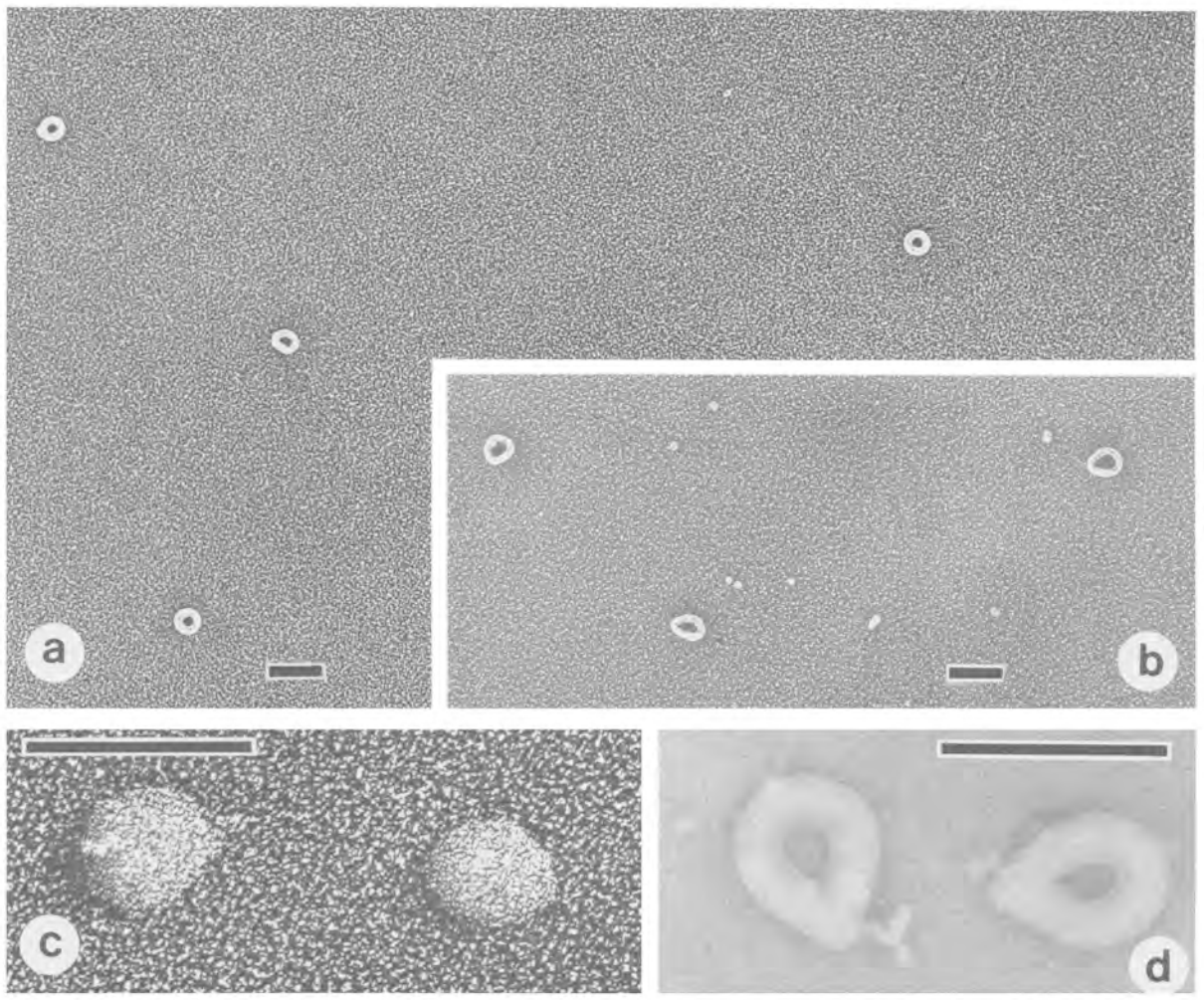

Figure 8. DNA Collapsed into Folded-Fiber Toroidal Forms

(a) λ DNA in 0.3 mM spermidine·3 HCl, 1 mM Tris (pH 7.5) immersed in 95% ethanol; (b) λ DNA in 3 mM $MgCl_2$, 1 mM Tris (pH 7.5) collapsed with 95% ethanol; (c) PM2 DNA toroids collapsed as in (a), but not immersed in 95% ethanol; (d) λ DNA toroids collapsed as in (a), but negatively stained to demonstrate the mechanism of toroid formation. It can be seen that toroids form by the fusion of the ends of bent rods. Bars = 2000 Å.

molecules scored as toroids are not in the true toroidal arrangement. As shown in Figure 8c, the DNA appears to be in flatter, uniformly spherical pools that have little or no central cavity. Our classification of these structures as toroids is based on the finding that the same percentages of the completely collapsed molecules scored as pools in the absence of an ethanol immersion (80%) can be scored as toroids in the presence of a 95% ethanol immersion (78%).

It is important to remember from the previous section that 95% ethanol brings about a tighter packaging of the DNA in polycation-induced rods. Assuming that the 95% ethanol immersion brings about a similarly tighter packaging of the DNA helices in the spermidine-collapsed DNA toroids, their retention of the toroidal configuration (that is, their failure to spread into spherical pools) would be explained. Gosule and Schellman (1976) have reported toroidal forms for spermidine-collapsed T7 DNA, indicating that the presence of an internal cavity depended upon their use of uranyl acetate staining. Toroidal structures with distinct internal cavities have also been reported with polylysine-collapsed DNA (Haynes et al., 1970); Olins and Olins, 1971; Laemmli, 1975). In all these cases, however, the collapsed molecules were either stained with uranyl acetate or immersed in alcohol before the final drying of the collapsed structures on the grid.

A question that remains to be answered is how the DNA is arranged in these toroidal structures. Is the DNA really in a conformation different from that of the folded-fiber rods that are forming at the

same time in the collapsing solutions? The formation of the two DNA toroids is shown in Figure 8d. Clearly these toroids are forming through the bending of the DNA rods and the subsequent fusion of their ends. The percentages of the DNA collapsed by spermidine (followed by 95% ethanol immersion) into toroidal and rod forms were determined for the five types of DNA molecules of increasing molecular weight used in this study. From this, the relative abundance of rods to toroids was computed to be: SV40, 1.8; PM2, 3.5; pDmre60, 5.8; λ, 11.1; and T5, 18.2. Unexpectedly, collapsed DNA molecules of high molecular weight have a greater tendency to form toroids than do the thinner rods made from lower molecular weight DNA. This suggests that rods of collapsed DNA are not rigid structures, and that the increased energy required to bend a thicker rod is more than compensated for by the energy derived from the increased area of DNA fusion at the ends of the rods. The controlling factor in the formation of toroids is probably the degree to which the DNA at the ends of the rods rejects the alcohol phase.

Discussion

The results of this study indicate that hydrated DNA can be collapsed into a variety of compact structures when subjected to alcohol dehydration. The manner of this collapse is determined by the extent of the concerted charge neutralization and dehydration of the DNA helix. Basically there are only *two qualitatively different* arrangements of the DNA helix within these structures—that of a supercoil (coiled helix) and that of a folded fiber (folded helix). Dehydration of DNA from solutions with little or no charge shielding (1 mM Tris) yields fibrillar cylindrical structures in which the DNA helix is coiled about the axis of the cylinder. This coiling of the DNA helix is not the same as the first-order supercoils (superhelical turns) normally found in a native covalently closed-circular DNA molecule (for example, mitochondrial DNA). There the supercoil is a plectonemic structure (Greek plectonemic = of interwoven threads) resulting from the interwinding of two DNA helices (from the two sides of the circle) about each other. We interpret the coiling found in our compact structures (Figure 3) as the result of one DNA helix continuously bending its axis in space much like a telephone cable. We define this as homonemic coiling (Greek homonemic = of same thread). We propose that this homonemic coiling is generated by a change in the turn-angle between consecutive base pairs of the double helix in such a manner that the conformation of the helix inside this supercoil is more like the C than the B form of DNA. We reached this conclusion when we found that covalently closed-circular DNA produced cylindrical fibers very similar in dimensions to those produced by their linear DNA counterparts. A covalently closed-circular DNA molecule can be compacted in this coiled form if the turn-angle between consecutive base pairs in the helix is increased—that is, if the right-handed helix becomes overwound. This overwinding of the helix brings a reduction in the number of base pairs per helix repeat length. Indeed it has been suggested on the basis of CD studies (Girod et al., 1973) that upon addition of alcohol, DNA in aqueous solutions changes from the B to the C form. From the results of several X-ray diffraction studies, it is known that DNA in the B form has 10 base pairs per turn and a rise per residue of 3.4 Å, while in the C form, there are 9.3 base pairs per turn and a rise per residue of 3.32 Å (Von Hippel and McGhee, 1972). The transition from the B to the C form then is accompanied by a change in turn-angle (screw-angle) from 36° to 38.7°—that is, an overwinding by 2.7°. The topological result of such an overwinding could be the cylindrical fiber (homonemic supercoil) reported here. Although it is not possible at this time to determine whether the DNA in our homonemic supercoil is indeed in the C form or another form very similar to that, we can definitely say that the DNA double helix is overwound. Starting with a covalently closed-circular DNA, this overwinding of the right-handed double helix can be accommodated by the generation of the left-handed homonemic supercoil, which in turn is topologically interconvertible with a right-handed plectonemic supercoil (Vinograd, Lebowitz and Watson, 1968).

A qualitatively different type of compaction is obtained when fully charge-shielded DNA is dehydrated. While alcohol dehydration is necessary for collapse when simple salts are used to neutralize the DNA, polycations induce rod formation in the absence of alcohol. Uniform rod-shaped structures approximately 2000 Å in length are formed regardless of the molecular weight of the DNA used. The cross-sectional area of these rods increases in a manner directly proportional to the DNA molecular weight. From this, we suggest that the DNA inside these rods is arranged as a folded fiber similar to that found in crystals grown from solutions of simple polymers (Keller, 1968). DNA collapsed into folded-fiber rods does not, however, always remain in such a configuration; DNA rods frequently bend, fusing their ends to form toroidal structures. The percentage of the rods that become toroids depends upon both the molecular weight of the DNA collapsed and the cation that has been used to shield the DNA charge.

In a previous electron microscopic study, Lang

(1973) has suggested that the DNA helix can enter into three consecutive orders of supercoiling—primary, secondary and tertiary. In this report, we have found evidence for only a "primary coiling" of the DNA (Figure 3). Both the pitch and diameter of this primary coiling vary considerably, as reflected in the degree of DNA compaction. DNA is compacted 4.2 fold in 10% ethanol, 5.4 fold in 95% methanol and 8.6 fold in 95% ethanol. The 30% ethanol structures described by Lang resulted in an 18 fold compaction of the DNA. While he describes this compaction as "secondary coiling," no evidence is presented that would exclude the possibility that this structure represents merely another form of primary supercoiling (for example, primary supercoils of different pitch and diameter). Finally, as we have mentioned previously, the "tertiary supercoiled" structures described by Lang are in fact the rods seen in the present study (Figure 7), in which we have clearly shown that the DNA helix is in a folded-fiber arrangement and is not supercoiled. This conclusion is in agreement with that which Lerman et al. (1976) derived from their earlier crystallographic studies of DNA crystalized from solutions of ethanol and 0.5 M sodium acetate.

The two major types of DNA compaction reported in this study are similar to published micrographs of compacted DNA found in a variety of living systems. We therefore believe that the in vitro DNA compaction elicited by the use of alcohol and salts may be under the control of physicochemical principles similar to those that control the compaction of DNA in vivo.

While electron microscopic studies have revealed the toroidal arrangement of the DNA inside certain bacteriophages and animal viruses (Comings, Chapman and Delong, 1965; Klemenko, Tikchonenko and Andreev, 1967; Furlong, Swift and Roizman, 1972; Richards, Williams and Calendar, 1973), the actual driving force behind this compaction is not known. As shown in the present report, any cation or polycation can spontaneously collapse DNA into folded-fiber (toroidal) forms provided the separation of a DNA phase occurs. This raises the possibility that spermine and spermidine are responsible for viral DNA compaction in vivo, since these polyamines are normally found in the host cell as well as in the mature virions (Ames and Dubin, 1960; Gibson and Roizman, 1971), are capable of bringing about the separation of a DNA phase in the absence of any other inducement (for example alcohol), yet have a low enough association constant with DNA to make their binding an easily reversible process. While more complex mechanisms have been suggested for the compaction of DNA inside a phage capsid (Laemmli, 1975), the packaging mechanism proposed here would be a very simple process requiring only that the polyamines already present in the cell associate with the phage DNA compacting inside an empty capsid. Once such a structure is completed and stabilized by intercapsomere interactions, there is no obvious need for the polyamines to remain associated with the DNA.

An electron microscopic approach to the problem of DNA packaging inside bacteria was recently undertaken by Griffith (1976), who reported that the lysis of bacterial cells directly onto hydrophilic carbon films results in the "beaded appearance" of the procaryotic DNA (similar to our Figure 1 or 5). It should be emphasized, however, that the micrographs published in that study showed DNA immersed in alcohol, and it remains to be shown whether the frequency and uniformity of the beads will remain unchanged when the potential for alcohol-induced condensation is eliminated. Furthermore, the direct lysis method precludes precise control of the microenvironment of the DNA being deposited on the grid, since the contents of the entire cell (crude lysate) are simultaneously deposited.

Most current models of chromatin, whether based upon X-ray diffraction or electron microscopic observations, suggest that the basic structure of chromatin is a fiber of closely adjacent beads approximately 100 Å in diameter. We have shown here that the DNA helix has an inherent tendency to compact by forming a left-handed homonemic supercoil when water is removed from its molecular domain. This raises the possibility that histones can accomplish a similar collapse of the helix by expelling water from the DNA grooves, due to their combined electrostatic and hydrophobic characteristics. It should be emphasized, however, that a homonemic supercoil of pure DNA exhibits an extensive flexibility and by itself would be an unstable statistical structure continuously modulated by random changes in the properties of its microenvironment, thus lacking the potential for localized regulation of its compaction state. If in chromatin, however, DNA-histone interactions modulate the supercoiling of the DNA, then histone-histone interactions could add stability to the supercoil and provide the chemical basis for an ordered, biologically meaningful control of that structure.

We have proposed a model for chromatin structure that is based on such a supercoil, two turns of which, stabilized by histone-histone interactions, are locked together to form the nucleosome or bead (Moudrianakis et al., 1977). Based on the results of the present study, we further propose that inside the approximately 100-Å chromatin fi-

ber, the DNA is compacted in a left-handed homonemic supercoil. Within this supercoil, the double helix is overwound relative to its state in the B form of DNA and thus more closely resembles the C form of DNA.

Experimental Procedures

DNA Preparations

Form I (supercoiled) and form III (linear) preparations of SV40 DNA were gifts from Dr. Thomas Kelley (Johns Hopkins University). The form I preparation was composed of over 93% supercoiled molecules. The form III preparation had been prepared by the treatment of form I DNA with the enzyme Bag 1 (Wilson and Young, 1976). PM2 phage DNA was a gift from Dr. Steve Rogers (Johns Hopkins University). This DNA was composed of approximately equal amounts of linear and open circular DNA which had been separated from form I DNA on a hydroxyapatite column (Bernardi, 1971). Form I and form III preparations of plasmid pDmre60 DNA were prepared by Dr. Igor Dawid following the NIH Guidelines under EKIP2 conditions. The form I preparation was composed of 82% supercoiled molecules. The form III DNA was derived from the treatment of form I DNA with the restriction enzyme Bam. The molecular weight of pDmre60 DNA is approximately 10.6×10^6 daltons (Dawid and Wellaur, 1977). Linear DNA from the phage λ was prepared as described previously (Rubin and Moudrianakis, 1972). Linear DNA from the phage T5 was a gift from Dr. Marcus Rhoades (Johns Hopkins University).

DNA Adsorption

Carbon-coated electron microscope grids were prepared by the slow evaporation of carbon onto freshly cleaved mica. These films were subsequently floated off the mica and onto copper grids precoated with a thin layer of polybutane, dried and stored in a desiccator. For DNA adsorption, 0.1 ml aliquots of DNA (0.2–2.0 μg/ml) in 1 mM Tris (pH 7.5) and with the various salts used in this study were placed as drops on a piece of Parafilm. Carbon-coated grids were touched to the drops and removed perpendicular to the solution surface so that a small amount of liquid adhered uniformly to the face of each grid. Grids were quickly plunged facedown in 95% ethanol for 10 sec, removed and allowed to dry faceup on filter paper. With this procedure, DNA collapse was found to be completely reproducible. In cases where DNA previously collapsed in solution was to be adsorbed to carbon films (for example, polylysine-collapsed DNA), grids made hydrophilic by the glow-discharge method were frequently used due to their better adsorption of DNA from solution.

Electron Microscopy

For routine observation, shadow casting was carried out with platinum at a 10:1 angle. To overcome the need for correction due to the thickness of the metal layer deposited in shadowing, however, *all measurements were obtained from negatively stained preparations*. Grids after adsorption and collapse of the DNA were allowed to air-dry for at least 10 min before immersion into a 2% uranyl acetate solution in 95% methanol for 5 sec and again allowed to air-dry. If the grids were allowed to air-dry completely before this staining routine, no changes in the collapsed structures could be detected after this additional wash.

Grids were examined in a JEM-100B electron microscope. For determination of the percentage of the DNA in each of the various collapsed conformations, the first 250 molecules observed on the grid were counted and scored accordingly. For the determination of the dimensions of the collapsed structures, 25–50 representative DNA molecules were photographed at a 20,000–50,000 direct magnification, projected onto a screen and measured. Absolute magnification was monitored with an optical grating calibration grid (54,800 lines per inch; Ted Pellco Company).

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