

# A simple model of DNA superhelices in solution

(supercoiled DNA/DNA topology/linking number/DNA conformation)

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**ABSTRACT** Closed circular DNA molecules in aqueous solution take the form of interwound superhelices over a wide range of superhelix densities. We describe a very simple model of such a superhelix in which twisting and bending forces are in balance, subject both to topological constraints and to a limitation on the distance of closest approach of the interwound duplexes of the superhelix. The model is consistent with some of the observed physical properties of closed circular DNA, and suggests that there may be severe limits to the range of allowable geometries for the superhelix structure of minimum energy.

The effect of supercoiling on the shape of covalently closed circular DNA molecules has been studied extensively by physicochemical methods and by electron microscopy. The data suggest that circular DNA molecules such as those isolated from simian virus 40 (SV40) take the form of interwound superhelices over a considerable range of variation in the topological winding or linking number (1–3). These interwound superhelices are probably not perfectly rodlike, but form a somewhat more compact structure either by branching (4, 5) or by generation of higher orders of supercoiling (6–8). Some experimental data (3, 9) are available concerning the dependence of the shape of supercoils on their linking number. However, as Upholt *et al.* (3) point out, no theoretical analysis of the shape as a function of linking number is possible “in the absence of some way of specifying the variation of the pitch and the radius of the interwound superhelix with superhelix density.”

In this paper, we investigate methods of specifying this variation for a very simple rodlike interwound helical structure. We assume, following the suggestion of Fuller (10), that the free energy of supercoiling contains contributions from bending and twisting forces of the DNA double helix, and that the equilibrium conformation is determined by the balance of these forces, subject to constraints on the minimum diameter of the superhelix. Given these assumptions, we are able to show that there are severe restrictions on the possible values of the number of superhelical turns, the twist and the pitch angle. The model also leads to the prediction that the sedimentation behavior should be nearly independent of the superhelical density. This is consistent with the experimental observation that in the range of linking number values near those found for SV40 DNA isolated from virions, the measured values of the sedimentation coefficient are practically invariant.

## RESULTS

The topological properties of covalently closed circular DNA molecules have been the subject of numerous studies (10–13). It is well known that such structures can be described in terms of their topological winding number, also termed the linking number,  $Lk$ . This number is an integer, and is invariant with

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respect to all distortions that do not break covalent bonds of the DNA chain. Methods for calculating the linking number of a structure have been described (12, 13).

In this discussion we will follow the approach of Fuller (10), further elaborated by Crick (13). The method permits direct introduction of helix geometry into the calculation. Fuller has pointed out that  $Lk$  can be expressed as the sum of the twist,  $Tw$ , and the writhing number,  $Wr$ :

$$Lk = Tw + Wr. \quad [1]$$

The writhing number is determined only by the shape of the space curve formed by the axis of the double helix. Because the twist of a simple ribbon structure is easily calculated,<sup>†</sup> it is also easy to deduce (10, 13) the writhing number of such a structure from the values of  $Lk$  and  $Tw$  by using Eq. 1. The value of  $Wr$  thus calculated will be correct for all structures based on the same space curve, i.e., all structures in which the axis of the double helix follows the same path. As Fuller has pointed out, the writhing number is not the same as the superhelix winding number defined in other analyses of this problem. In the following analysis of superhelical DNA, it is also important to remember that in general neither  $Lk$  nor  $Wr$  will equal  $N$ , the number of superhelical turns.

**Interwound Superhelix.** For illustrative purposes, we will use SV40 DNA, which has an average value of  $\Delta Lk$  of about  $-26$  when isolated from virions (14). Hydrodynamic and electron microscopic studies show that SV40 molecules with values of  $\Delta Lk$  in the approximate range  $-18$  to  $-40$  exist in solution as interwound superhelices, rather than in more open (e.g., toroidal) forms. Furthermore, there is very little dependence of the sedimentation coefficient of SV40 on  $\Delta Lk$  in this range:  $s_{20,w}$  (measured in 1 M NaCl) has the value  $21.8 \pm 0.6$  S.

We have calculated the energy of formation of various interwound superhelical structures. The free energy of such a structure, neglecting all other sources and ignoring cross terms, is comprised of a contribution from DNA bending, and another from twisting:

$$\Delta G = \Delta G_B + \Delta G_T \quad [2a]$$

Abbreviation: SV40, simian virus 40.

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<sup>†</sup> We refer the reader to the paper by Crick (13) for definitions and methods of calculating the twist and linking number. In this paper we consider deviations from the fully relaxed closed circular form of DNA. The actual value of  $Tw$  for the relaxed structure depends upon temperature and solvent conditions. The value of  $Wr$  for the relaxed structure is close to zero, and we set it equal to zero. Deviations from the relaxed form can thus be expressed as

$$\Delta Lk = \Delta Tw + Wr. \quad [1a]$$

$\Delta Tw$  is assumed positive when right-handed, i.e., when the duplex is overwound.

$\Delta G$  is assumed to be negligible for the relaxed closed circular form. The value of  $\Delta G$  has been estimated experimentally, both from the details of the Boltzmann distribution of linking numbers about the relaxed state (15, 16) and from ethidium bromide titration data (17). We can approximate the data by the equation

$$\Delta G_e = A(\Delta Lk)^2, \quad [2b]$$

in which  $\Delta G_e$  is the experimental value of  $\Delta G$ , and we have used 0.1154 kcal/mol (1 cal = 4.184 J) as the value of  $A$  for SV40 DNA. Although a more exact equation would contain a cubic term as well (17), the inclusion of such a term has a negligible effect on our results. The free energy of bending can be expressed (18–20) as

$$\Delta G_B = BK^2S \quad [3]$$

in which  $B$  is one-half the force constant for bending,  $K$  is the curvature (reciprocal of the radius of curvature), and  $S$  is 18,270 Å, the total contour length of SV40 DNA [assuming a molecular weight of  $3.6 \times 10^6$  (16)]. All the structures we consider are uniformly bent, i.e., we assume that the superhelix has a uniform pitch, and that negligible contributions are made by the loops at the ends, where the superhelix reverses direction. [We have considered the effect of such loops in detail (unpublished calculations). They have little influence on the results.] The experimental value of  $B$  can be estimated either from the persistence length of DNA (18) or from the temperature dependence of the persistence length (20). The methods respectively give about 180 and 128 kcal-Å/rad<sup>2</sup>-mol at 20° as values of  $B$ . We choose the latter value, but the results below do not depend strongly on the choice.

We will assume that the free energy of twisting can also be expressed (10) in quadratic form:

$$\Delta G_T = CS(\Delta Tw/S)^2 = C(\Delta Tw)^2/S. \quad [4]$$

$\Delta G_T$  is proportional to the square of the twist per unit length and the first power of the length;  $C$  is a force constant for which no independent experimental estimates exist.

For any given value of  $\Delta Lk$ ,  $\Delta Tw$  and  $Wr$  are related by Eq. 1. Because the curvature of the superhelix is related to the writhing number, not all the parameters in Eqs. 3 and 4 are independent variables. To calculate the writhing number of a right-handed interwound helix, we consider a ribbon wrapped smoothly on the surface of a cylinder (13) to make  $N$  complete interwound right-handed superhelical turns ( $N/2$  up the cylinder, and  $N/2$  down again). Assuming that the ascending and descending sections are joined by negligible, twist-free segments, the linking number of this structure is 0 and the twist is  $N \sin \alpha$ , in which  $\alpha$  is the pitch angle of the helix (10, 13). The writhing number, from Eq. 1, is  $-N \sin \alpha$  (note that  $N$  is always positive), and this will be the value of  $Wr$  for all right-handed interwound superhelices with  $N$  superhelical turns of pitch angle  $\alpha$ , regardless of the values of  $Lk$  and  $Tw$ . Thus, for an interwound DNA superhelix,

$$\Delta Tw = \Delta Lk + N \sin \alpha. \quad [5]$$

Let us suppose that our interwound helix has pitch  $p$  and superhelical radius  $r$ . The curvature of a regular helix (10) is given by  $K = 4\pi^2r/(4\pi^2r^2 + p^2)$ , and  $\sin \alpha = Np/S$ . We note further that  $S^2 = 4\pi^2N^2r^2 + N^2p^2$ . Substituting these values in Eqs. 2–5, we find

$$\Delta G = \frac{4\pi^2BN^2}{S^3}(S^2 - x^2) + J \left( \Delta Lk + \frac{Nx}{S} \right)^2. \quad [6]$$

in which  $J = C/S$  and  $x = Np$ . The free energy of the interwound superhelix with linking number  $\Delta Lk$  is thus expressed

in terms of the number of superhelical turns,  $N$ , and  $x$ , which is the product of  $N$  and the pitch of the superhelix.

We point out again that the value of  $N$  is not necessarily equal to the absolute value of  $\Delta Lk$ , or to the writhing number. The actual values of  $N$  and  $p$  must be determined by the balance between the forces of bending and twisting. We might naively expect to determine the equilibrium point by minimizing  $\Delta G$  with respect to variation in  $N$  and  $x$ . As Fuller (10) has pointed out, however, this minimization has only a trivial solution. The idealized interwound superhelix can always find a lower energy by increasing  $\alpha$  and decreasing  $r$ , thus reducing both curvature and twist simultaneously. Fuller has also suggested the solution to this anomaly: The backbone of the superhelix occupies space, and unfavorable contacts provide a limit to distances of approach between its elements, so that we need only consider those structures in which this limit is achieved. Under such constraints, a minimum in the free energy does exist.

A very simple way to approximate this constraint is to require that the radius of the superhelix be fixed at some minimum value  $r_0$ . Given the dimensions of the structures to be discussed below,  $2r_0$  can be thought of as a distance of closest approach of the ascending and descending arms of the superhelix (in this model we assume that the two arms are related by a 180° rotation axis along the cylinder axis). Using the method of Lagrange multipliers, we can minimize  $\Delta G$  (Eq. 6) with respect to variation in  $N$  and  $x$ , subject to this constraint. The solution to this problem exists, and can be shown to be a minimum (see *Appendix*).

A surprising amount of information can be extracted from these results. We first note that the solution requires (*Appendix*, Eq. i)

$$J = \frac{ENx(S^2 - x^2)}{\left( \Delta Lk + \frac{Nx}{S} \right) (S^2 - 2x^2)} \quad [7]$$

in which  $E$  is a positive constant. Because  $J$  by its nature must be positive, solutions are restricted<sup>‡</sup> to the range  $x > S/\sqrt{2}$  and  $N < |\Delta Lk|\sqrt{2}$ . Furthermore,  $\Delta Tw$  (given by Eq. 5) must be negative (i.e., the duplex is underwound).

What is the upper limit to  $N$ ? We have already shown that  $N$  is certainly no larger than  $|\Delta Lk|\sqrt{2}$ , but another consideration intervenes. From the preceding argument, we know that both terms in the denominator of Eq. 7 are negative. Because  $\Delta Lk$  is negative, the term  $(\Delta Lk + Nx/S)$  can be negative only if  $Nx/S < |\Delta Lk|$ . The maximum value of  $Nx/S$  thus occurs at  $\Delta Lk + Nx/S = 0$ , i.e., when  $\Delta Tw = 0$ . At that point, characterized by some value of  $x$  which we call  $x_0$ , the free energy is entirely due to bending, so that

$$\Delta G = \Delta G_B = A(\Delta Lk)^2 = 4\pi^2BN^2(S^2 - x_0^2)/S^3. \quad [8]$$

<sup>‡</sup> Because  $S > x$ , the numerator of Eq. 7 is positive; because  $J$  is positive, the denominator must also be positive. The two factors in the denominator are therefore either both positive or both negative. For negative values of  $\Delta Lk$  this condition is met in two regions: either  $x < S/\sqrt{2}$  and  $N > |\Delta Lk|\sqrt{2}$ , or  $x > S/\sqrt{2}$  and  $N < |\Delta Lk|\sqrt{2}$ . It is also evident that the bending free energy  $\Delta G_B$ , given by the first right-hand term of Eq. 6, cannot exceed the total experimental energy of supercoiling (Eq. 2b). This means that  $A(\Delta Lk)^2 \geq 4\pi^2BN^2(S^2 - x^2)/S^3$ ; substituting appropriate numerical values for SV40 DNA, we find that when  $x = S/\sqrt{2}$ ,  $N \leq 0.914|\Delta Lk|$ . If  $x < S/\sqrt{2}$ ,  $N$  has a still smaller upper limit. Because we showed above that for  $x < S/\sqrt{2}$ ,  $N$  must exceed  $|\Delta Lk|\sqrt{2}$ , it follows that no solutions exist in this range of  $x$  values. We have thus shown that  $x$  has a lower limit at  $S/\sqrt{2}$ . This lower limit arises from the fact that we have considered only positive values of  $N$ , corresponding to right-hand interwound supercoils. Experimental evidence (17) supports the assumption that in aqueous solution  $N > 0$  when  $\Delta Lk < 0$ .

Imagine that we now move to a new equilibrium configuration (with a different assumed value of  $J$ ) characterized by a value of  $x$  less than  $x_0$ . In that case, the term  $(S^2 - x^2)$  in the expression for  $\Delta G_B$  increases, and because  $\Delta G_B$  can never exceed  $A(\Delta Lk)^2$ ,  $N$  must decrease. Similarly, if we choose  $x > x_0$ ,  $N$  must also decrease, because  $Nx/S$  has its maximum value at  $x_0$ . Thus, the maximum allowed value of  $N$  occurs when  $\Delta Tw = 0$ . It is easy to evaluate the upper limit of  $N$  using Eq. 8. We find that  $N \leq 1.19 |\Delta Lk|$ . There is no positive lower limit to  $N$ .

The energy minimization conditions also lead to the equation (Appendix)

$$N = \Delta LK \left\{ -\frac{x}{S} \pm \frac{1}{2} \left[ \frac{4x^2}{S^2} + \frac{AS}{\pi^2 B} \left( \frac{S^2 - 2x^2}{S^2 - x^2} \right) \right]^{1/2} \right\}. \quad [9]$$

Because  $N$  is real, the term under the square root sign must be  $> 0$ . Substituting the known values of the parameters, we find that this is equivalent to requiring  $x \leq 0.857S$ . (Note that the configuration with maximum value of  $x$  does not have the maximum value of  $N$ .) We showed earlier that  $x \geq 0.707S$ ; the range of possible values of  $x$  is thus severely restricted. Because the sine of  $\alpha$ , the pitch angle, is  $x/S$ , we conclude that the pitch angle is restricted to the range between  $45^\circ$  and  $59^\circ$  for interwound superhelices satisfying the energy minimization conditions, regardless of the values of any other parameters.

Within the limits we have described, the parameters are unrestricted in value, because we have one more unknown than we have equations. In principle, it is only necessary to specify a value for  $r_0$  or  $J$  to determine the shape of the minimum energy superhelix completely.

We first consider the class of solutions in which the value of  $J$  is assumed to be constant. Solutions to the minimization equations are shown in Fig. 1A, in which the value of  $r_0$  is plotted against the chosen value of  $J$  to which it corresponds, for various values of  $\Delta Lk$ . Values of the parameters for the minimum energy superhelices with  $J = 2$  and with  $J = 10$  are given in Table 1A. Note that  $r_0 \Delta Lk$  and  $Np$  are constant for a fixed  $J$ . Because  $Np (= x)$  is twice the length of the superhelical "rod," the length of this rod is independent of  $\Delta Lk$  for a chosen value of  $J$ .

If the superhelical radius  $r_0$  represented a physically impenetrable barrier, we might expect that  $r_0$  would be a constant, independent of  $\Delta Lk$ . It is therefore of interest to explore a second class of solutions, in which  $r_0$  is fixed for all  $\Delta Lk$  and the parameter  $J$  is allowed to vary.

The assumption of a variable  $J$  is physically reasonable; the twist force "constant" might be a function of  $Tw$  or  $Wr$ . We have no way at present of measuring or calculating the detailed dependence of  $J$  on these parameters, but we can examine the properties of a simple model in which  $r_0$  is fixed and  $J$  is assumed to be some function of  $\Delta Lk$ . The solutions to this problem are identical to those shown in Fig. 1A, and the limits on  $N$  and  $x$  still exist. The data in Fig. 1B show the dependence of  $J$  on  $\Delta Lk$  for various assumed values of  $r_0$ . It should be noted that the curves in Fig. 1B could have been obtained in principle from the curves in Fig. 1A. For any given  $r_0$ , there is a lower limit to the value of  $|\Delta Lk|$ .

The properties of the minimum energy superhelices at fixed  $r_0$  are given in Table 1B. There is very little variation in  $N$  or  $x$ ; most of the change in  $\Delta Lk$  is accommodated by a change in  $\Delta Tw$ . Once again, the rod length is relatively constant. It can be shown that this behavior is consistent with a free energy of twist containing terms both quadratic and linear in  $\Delta Tw$ . It should be noted that for  $r_0 = 150$  (as well as for solutions at larger values of  $r_0$ ),  $J$  is in fact nearly invariant with  $\Delta Lk$ .

**Variation in  $\Delta G$  with Conformation.** How does the free

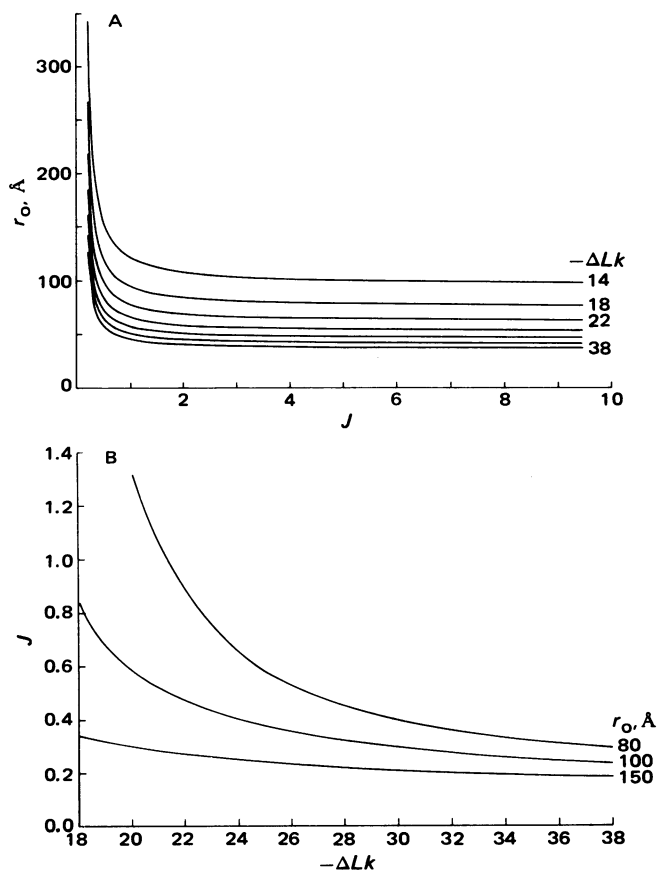


FIG. 1. (A) Dependence of  $r_0$  on  $J$  for various values of  $\Delta Lk$ . Adjacent curves correspond to an interval of 4 in the value of  $\Delta Lk$ . (B) Dependence of  $J$  on  $J$  for various values of  $r_0$ .

energy vary with excursions about the equilibrium configuration? We have chosen a "typical" equilibrium configuration of SV40 DNA ( $\Delta Lk = -26$ ,  $J = 1.98$ ,  $r = 57.9 \text{ \AA}$ ,  $N = 26.1$ ) and calculated the variation in  $\Delta G$  with  $N$  (Eq. 6) about this configuration, with  $r$  held constant. We find that the dependence of  $\Delta G$  on  $N$  is quadratic in the range  $\Delta N = \pm 1$  around the equilibrium value, and that  $\Delta N = \pm 1$  corresponds to a free energy increase of about  $2RT$  per mol of SV40 DNA. The range of predicted excursions about the equilibrium configuration is thus quite limited.

**Physical Studies.** It was noted by Upholt *et al.* (3) that the sedimentation coefficient of SV40 DNA varies only slowly with linking number over a considerable range of values of  $\Delta Lk$  ( $\sim -18$  to  $-40$ ). The dependence of sedimentation coefficient on  $\Delta Lk$  can be calculated for our simple model. The parameters presented in Table 1 define a family of long, relatively thin rods. The sedimentation properties of such rods depend principally upon their length, which has the value  $x/2$ . Because  $x$  is invariant with  $\Delta Lk$  for the set of solutions in Table 1A (corresponding to  $J$  constant), and varies very little for the solutions in Table 1B ( $r_0$  constant), we expect that the predicted values of  $s_{20,w}$  will be nearly invariant with  $\Delta Lk$  for either set of solutions.

This conclusion is substantiated by direct calculation of the frictional coefficient of our model structures. The frictional coefficient of an interwound superhelix has been calculated by Gray (1), using the Kirkwood-Riseman equations (21), and replacing the DNA double strand by an equivalent string of contiguous spherical beads. The bead diameter is taken as  $27.2 \text{ \AA}$ , approximately the effective hydrodynamic diameter of a

Table 1. Calculated parameters of SV40 DNA superhelix of minimum energy

(A) Constant $J$									
$J = 2$					$J = 10$				
$-\Delta Lk$	$N$	$r, \text{\AA}$	$p, \text{\AA}$	$s_{20,w}, S$	$N$	$r, \text{\AA}$	$p, \text{\AA}$	$s_{20,w}^*, S$	
18	18.1	83.5	861	17.3	20.5	75.6	752	17.6	(22.0)
22	22.1	68.3	705	17.7	25.1	61.9	615	18.0	(22.3)
26	26.2	57.8	596	18.1	29.7	52.4	521	18.4	(22.0)
30	30.2	50.1	517	18.4	34.2	45.4	451	18.7	(21.8)
34	34.2	44.2	456	18.6	38.8	40.0	398	18.9	(21.6)
38	38.2	39.6	408	18.8	43.4	35.8	356	19.2	(21.4)
40	40.3	37.6	388	19.0	45.6	34.0	338	19.3	(21.2)

(B) Constant radius									
$r_o = 150 \text{\AA}$					$r_o = 80 \text{\AA}$				
$-\Delta Lk$	$N$	$x, \text{\AA}$	$Wr$	$\Delta Tw$	$N$	$x, \text{\AA}$	$Wr$	$\Delta Tw$	
18	10.30	15,475	-8.73	-9.27	19.07	15,554	-16.2	-1.77	
22	10.55	15,329	-8.85	-13.2	18.85	15,646	-16.1	-5.86	
26	10.77	15,191	-8.96	-17.1	18.87	15,616	-16.1	-9.87	
30	10.97	15,066	-9.04	-21.0	19.09	15,548	-16.2	-13.8	
34	11.14	14,952	-9.12	-24.9	19.34	15,470	-16.4	-17.6	
38	11.29	14,849	-9.18	-28.8	19.58	15,391	-16.5	-21.5	
40	11.36	14,802	-9.21	-30.8	19.70	15,353	-16.6	-23.4	

\* Values in parentheses are experimentally observed values for the given linking number (3).

double-stranded DNA; the results presented below do not depend critically on this value. To calculate the frictional coefficient, it is necessary to evaluate (21, 22) the sum  $\sum \sum (R_{ij})^{-1}$ , in which  $R_{ij}$  is the distance between beads  $i$  and  $j$ , and the sum extends over all pairs of beads.

The terms  $R_{ij}$  are calculated for all pairs directly from the coordinates of the beads. Values of the calculated sedimentation coefficients are shown in Table 1A. It is evident that regardless of the value of  $J$  chosen, the sedimentation coefficient varies only slowly with linking number, a result consistent with experimental observation (3). The predicted value of  $s_{20,w}$  for  $\Delta Lk = -26$  and  $J = 2$  or 10 is about 17% lower than the experimental value. Similar calculations could be carried out for the data in Fig. 1B;  $s_{20,w}$  would be somewhat smaller, but it would also be invariant with  $\Delta Lk$ .

Although the simple model we are analyzing leads to reasonable predictions of the behavior of the sedimentation coefficient, the model is not sufficient to account for other known properties of superhelical molecules in solution. For example, the radius of gyration (5) of supercoiled SV40 DNA isolated from virions is about 930 Å, while a rodlike interwound supercoil of the dimensions we are considering would have a radius of gyration a little more than twice as large. Real supercoiled molecules are thus somewhat more compact than our rodlike ideal.

It has been suggested that this compaction can be accounted for either by branching of the interwound superhelix (4, 5), or by the presence in the interwound superhelix of a second order of supercoiling (6-8). Either model can be made reasonably consistent with all of the data (unpublished calculations) because each of these perturbations of the simple rodlike structure has a larger effect on the radius of gyration than on the frictional coefficient.

### DISCUSSION

We have considered a simple model of the forces governing superhelix shape. Following Fuller's suggestion (10), we have

assumed that the structure is interwound and rodlike, and minimized the free energy contributions from twisting and bending, subject to a constraint on the radius of the superhelix. We are aware of limitations in this approach, even within the restrictions of this simple model.

We have calculated conformations of minimum energy subject to the constraint that there is a constant superhelix radius,  $r_o$ , for each value of  $\Delta Lk$ . If  $J$  is fixed and completely independent of  $\Delta Lk$ ,  $r_o$  is determined, and varies with  $\Delta Lk$  (Table 1A). As we have pointed out above, this does not seem physically reasonable if  $r_o$  is thought of as a constant distance of closest approach.

There are various ways of resolving this difficulty. For example, we can fix  $r_o$  and allow  $J$  to vary with  $\Delta Lk$ , with the results shown in Table 1B. Among these solutions there is a limiting set for large  $r_o$  (e.g.,  $r_o = 150 \text{\AA}$ ; Fig. 1B), for which  $J$  varies only slowly with  $\Delta Lk$ . For larger values of  $r_o$ , the variation is even smaller. Thus, in this region, all of the original assumptions of the model are nearly satisfied. It should be noted that the solutions in this range compare favorably with estimates of the dimensions of first-order interwound superhelices obtained from low-angle x-ray scattering measurements in solution (6, 7). The data for phage PM2 DNA are most consistent with a superhelical radius of 180-200 Å, and a pitch of 1400-1000 Å. We find that the minimum energy interwound superhelix with  $r_o = 180 \text{\AA}$  and  $\Delta Lk = -26$  has a pitch of 1640 Å.

Another possibility is to introduce in the equation for  $\Delta G$  an explicit dependence upon the superhelical radius. We find that if an energy term proportional to  $1/r^2$  is added to  $\Delta G$ , and  $\Delta G$  is minimized with respect to  $r$  as well as  $N$  and  $x$ , the resulting equation for  $\Delta G$  is of a general form similar to Eq. ii of the Appendix, with a solution of the form  $N = \Delta Lk \cdot f(x)$ . The solution for  $J$  (Eq. 7) is unchanged, so that  $x$  is invariant with respect to  $\Delta Lk$ . The general properties of the solutions given in Table 1A are thus preserved without assuming that  $r$  has a fixed value.

Benham (8) has pointed out that the most general solution to the problem of a uniform flexible rod undergoing deformation involves two orders of supercoiling, and low-angle x-ray scattering data alluded to above provide some evidence for such a structure (6, 7). It should be possible to extend the analysis presented here to include second-order superhelical perturbations.

Alternatively, it has been proposed (4, 5) that there may be distinct points in the supercoil at which local denaturation is favored, leading to the formation of a fixed number of interwound superhelical branches (three in the case of SV40 DNA) of fixed length. The extension of our analysis to such a structure is straightforward, because each interwound superhelical arm can be treated as a simple rod, independent of the other arms.

The most general conclusions that can be derived from our model concern restrictions on the parameters  $N$  and  $x$ . When the value of  $\Delta Lk$  of a closed circular DNA molecule is known, there is not enough information to determine the number of superhelical turns, the pitch of the superhelix, or the twist. To the extent that our assumptions are correct, limits can be placed on these parameters. The simplest model leads to the prediction that interwound superhelices at equilibrium and with  $\Delta Lk < 0$  have the following properties: (i) The number of superhelical turns cannot be greater than 1.2 times  $\Delta Lk$ . (ii)  $\Delta Tw$  must be negative or zero. (iii) The number of superhelical turns times the pitch is restricted to values between  $0.707 S$  and  $0.86 S$ , in which  $S$  is the DNA contour length. (iv) The pitch angle is between  $45^\circ$  and  $59^\circ$ .

It is known that certain combinations of histones induce supercoiling when bound to closed circular DNA (23–25), but, as we have pointed out elsewhere (24), the detailed geometry of DNA packing in nucleosomes must be quite different from the geometry of closed circular SV40 DNA described here.

All of our conclusions about superhelix geometry can be reached assuming only that  $\Delta G$  is composed of quadratic contributions from twisting and bending, that these are in balance under conditions of a fixed minimum superhelix radius, and that  $\Delta G$  must equal its measured experimental value. Some of the above restrictions do not depend on the exact experimental value of  $\Delta G$ . Although our model is simple, it permits us to think of the equilibrium form of a superhelix as arising from a balance of forces, and it can serve as a starting point for more elaborate analyses of the energetics of supercoiling.

#### Appendix: Free energy minimization with constraints

The free energy  $\Delta G$  is assumed to have the form given in Eq. 6 of the text. We now assume that the superhelix is wrapped around a cylinder of radius  $r_0$ , and that all deformations are subject to this constraint. This restriction has the form:  $g(N, x) = 4\pi^2 N^2 r_0^2 + x^2 - S^2 = 0$ . We define a function  $V = \Delta G + \lambda g$ , in which  $\lambda$  is a Lagrange multiplier. The minimization conditions are:  $(\partial V / \partial N)_x = (\partial V / \partial x)_N = 0$ . The equations are solved, after differentiation, by eliminating  $\lambda$ . Identical results are of course obtained by direct substitution of the expression for  $g(N, x)$  in  $\Delta G$  to eliminate either  $x$  or  $N$  before taking the

derivative with respect to the remaining variable.

We find

$$J \left[ \Delta Lk + \frac{Nx}{S} \right] = \frac{8\pi^2 BNx}{S^2} \left[ \frac{S^2 - x^2}{S^2 - 2x^2} \right]. \quad [i]$$

Substituting the expression on the left-hand side into Eq. 6 of the text, we obtain a quadratic equation for  $\Delta G$  in terms of  $N$  and  $x$ , which holds true only for the minimum-energy values of  $N$  and  $x$ ,

$$\Delta G = \left[ \frac{S^2 - x^2}{S^2 - 2x^2} \right] \left[ \frac{DS^2}{4\pi^2} \cdot N^2 + \frac{DSx \cdot \Delta Lk}{2\pi^2} \cdot N \right], \quad [ii]$$

in which  $D = 16\pi^4 B / S^3$ .

We now make use of text Eq. 2b,  $\Delta G_e = A(\Delta Lk)^2$ , set  $\Delta G = \Delta G_e$ , and solve the quadratic equation for  $N$  in terms of  $x$ . The solution is Eq. 9. By the use of second derivatives, it is possible to show that the solution is a relative minimum.

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