

## The Effect of Ionic Conditions on the Conformations of Supercoiled DNA. I. Sedimentation Analysis

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We studied the conformations of supercoiled DNA as a function of superhelicity and ionic conditions by determining its sedimentation coefficient both experimentally and by calculation. To cancel out unknown parameters from both calculations and experiments, we determined the ratio of the sedimentation coefficient,  $s$ , to that of open circular DNA,  $s_{oc}$ . Calculations of the sedimentation coefficient were based on direct solution of the Burgers-Oseen problem for an equilibrium set of DNA conformations generated for each condition by the Metropolis Monte Carlo procedure. There were no adjustable parameters in the Monte Carlo simulations because all three parameters of the DNA model used, bending and torsional elasticity of DNA and DNA effective diameter specifying electrostatic interactions, were known from independent data. The good agreement between measured and calculated values of  $s/s_{oc}$  allowed us to interpret the sedimentation results in terms of DNA conformations, with particular emphasis on the marked effect of ionic conditions. As NaCl concentration decreases,  $s/s_{oc}$  increases because the superhelix becomes less regular and more compact. In the presence of just 10 mM MgCl<sub>2</sub>, supercoiled DNA adopts essentially the same set of conformations as in moderate to high concentrations of NaCl. Our simulations showed that  $s$  is a strong function of the superhelix branching frequency. At near physiological ionic conditions, there are about four branches in the 7 kb DNA molecule used in this work. We found no indication of superhelix collapse in any ionic conditions even remotely approaching physiological ones. For all ionic conditions studied, we conclude that the electrostatic interaction of DNA segments specified by the DNA effective diameter is the primary determinant of supercoiled DNA conformations.

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### Introduction

Thirty years ago Vinograd and co-workers discovered the supercoiling of DNA (Vinograd *et al.*, 1965). Since that time, it has become clear that the DNA in virtually all organisms is supercoiled and that supercoiling is essential for the functions of DNA. Recently, the conformational aspects of DNA supercoiling have been analyzed by a variety of experimental and theoretical methods. The experimental studies principally employed electron microscopy, hydrodynamic measurements, and topological methods (Laundon & Griffith, 1988; Adrian *et al.*, 1990; Boles *et al.*, 1990; Bednar *et al.*, 1994; Langowski *et al.*, 1994), while the theoretical approaches involved either computer simulations (Hao & Olson, 1989; Klenin *et al.*, 1991; Schlick &

Olson, 1992a,b; Vologodskii *et al.*, 1992; Bauer *et al.*, 1993; Schlick *et al.*, 1994a,b; Tesi *et al.*, 1994; Gebe *et al.*, 1995; Klenin *et al.*, 1995; Vologodskii & Cozzarelli, 1995; 1996) or analytical treatment (Hearst & Hunt, 1991; Hunt & Hearst, 1991; Marko & Siggia, 1994, 1995; Shi & Hearst, 1994). As a result of these studies, many conformational properties of supercoiled DNA have been clarified (for a review see Vologodskii & Cozzarelli, 1994). We know that the linking number difference,  $\Delta Lk$ , the quantitative measure of DNA supercoiling, is distributed between the changing of the double helix twist,  $\Delta Tw$ , and the folding of the DNA axis, which is quantitatively specified by writhe,  $Wr$ . At physiological salt conditions, the ratio  $Wr/\Delta Tw$  is about equal to 3 and depends on neither supercoil-

ing density,  $\sigma$ , nor DNA length for DNA longer than 3 kb (Adrian *et al.*, 1990; Boles *et al.*, 1990; Vologodskii *et al.*, 1992; Vologodskii & Cozzarelli, 1994). The supercoiled DNA adopts interwound, or plectonemic, conformations, which are often branched, as determined by electron microscopy (Rhoades & Thomas, 1968; Laundon & Griffith, 1988; Adrian *et al.*, 1990; Boles *et al.*, 1990; Bednar *et al.*, 1994), Monte Carlo simulation (Klenin *et al.*, 1991; Vologodskii *et al.*, 1992; Vologodskii & Cozzarelli, 1994), and the analysis of the products of topoisomerases (Wasserman & Cozzarelli, 1991) and recombinases (Boles *et al.*, 1990). Electron microscopy studies determined that the average superhelix winding angle is about  $60^\circ$  and does not depend on  $\sigma$  (Adrian *et al.*, 1990; Boles *et al.*, 1990). An analysis of the products of site specific recombination demonstrated that within *Escherichia coli* cells, supercoiled DNA is plectonemic (Bliska & Cozzarelli, 1987; Bliska *et al.*, 1991).

Despite the good agreement between these basic conformational features of supercoiled DNA obtained by different techniques and different groups of investigators, for two aspects of supercoiling there are troubling differences between various experimental and computational data. The first concerns the branching of the superhelix. According to the simulation results, the average number of superhelix branches should be proportional to DNA length if the length is larger than 3 kb (Vologodskii & Cozzarelli, 1994; 1996) with a proportionality of one branch per 1.7 kb. The branching frequency measured by electron microscopy, however, differs greatly in different studies, with branching values both greater and lesser than one per 1.7 kb (Laundon & Griffith, 1988; Boles *et al.*, 1990).

The second discrepancy concerns the effect of ionic conditions on the diameter of the superhelix. Both experiments and theory indicate that ionic conditions are important determinants of the conformational properties of topologically constrained DNA (Klenin *et al.*, 1988; Adrian *et al.*, 1990; Hunt & Hearst, 1991; Klenin *et al.*, 1991; Vologodskii *et al.*, 1992; Rybenkov *et al.*, 1993; Shaw & Wang, 1993; Bednar *et al.*, 1994; Schlick *et al.*, 1994a; Tesi *et al.*, 1994). Recently, Dubochet and co-workers observed by cryoelectron microscopy (cryo EM) a dramatic effect of ionic conditions on conformations of supercoiled DNA. In the presence of at least 10 mM  $MgCl_2$  or 0.1 M NaCl, this group observed a collapse of the plectonemic superhelix (Adrian *et al.*, 1990; Bednar *et al.*, 1994), resulting in a structure in which there is no visible space between the opposing segments of the interwound superhelix. Thus, the superhelix diameter is just twice the double helix diameter. Because *in vivo* the concentrations of ions are higher than the threshold for collapse, they argued that the collapsed form of the superhelix is the physiologically relevant conformation of DNA. The collapse of the superhelix was never observed by conventional electron microscopy. Nor was it observed in com-

puter simulations of supercoiling because of thermal fluctuations and electrostatic repulsion between DNA segments. However, we have only limited knowledge about the interaction between DNA segments separated by very short distances. An attraction of DNA segments such as that provided by di- and polyvalent cations (Rau & Parsegian, 1992; Shaw & Wang, 1993; Ma & Bloomfield, 1994; Bloomfield, 1996) could conceivably change the simulation results enough to cause collapse of the superhelix.

These questions cannot be solved by theoretical analyses or by further EM studies alone. Theoretical analysis requires the use of a simplified model of DNA, so the results will depend on the initial choice of model. The major limitation of EM is that labile features of superhelix conformations may be substantially altered during sample preparation, and it is not known when during these manipulations the DNA is fixed in its final observed form (discussed by Vologodskii & Cozzarelli, 1994). Conventional electron microscopy is limited by sample deformation during changes in the solution composition, dehydration, staining, shadowing, and adhesion to the grid surface. In cryoelectron microscopy, it is not known if the cooling is rapid enough to prevent conformational changes, and strong surface forces are also clearly present and may deform the DNA. Therefore one needs additional methods in which the DNA is in true solution.

Hydrodynamic and optical methods have clear advantages, because they measure structure-dependent features in well-defined solutions while perturbing conformation only minimally. Sedimentation analysis, the first physical probe of supercoiled DNA conformations, showed that the sedimentation coefficient,  $s$ , increases with the absolute value of superhelix density,  $\sigma$ , reaches a maximum, and then slightly decreases to a local minimum (Wang, 1969, 1974; Upholt *et al.*, 1971). The maximum value of  $s$  is greater than that of open circular DNA by 30 to 60%, depending on DNA length. Since the sedimentation coefficient reflects an overall compactness of the molecule, these results showed clearly the range of  $\sigma$  in which the formation of superhelix structure takes place. However it was difficult to make additional structural conclusions from the data themselves. The value of such data is augmented greatly when combined with a theoretical analysis that allows a structural interpretation of the experimental results. This dual approach has been used in the study of the conformational properties of linear DNA molecules (for example, see review by Hagerman, 1988) and of short supercoiled DNA molecules (Langowski *et al.*, 1994).

The first attempt to apply a combined experimental and theoretical approach to supercoiled DNA was made 18 years ago by Camerini-Otero and Felsenfeld (1978). Only recently, however, have advances in computational opportunities allowed the statistical-mechanical analysis of supercoiled DNA

**Table 1.** Sedimentation coefficient of 7.0 kb open circular DNA at different ionic conditions

[Na <sup>+</sup> ](M)	Ionic conditions		Measured		Calculated
	[Mg <sup>2+</sup> ](M)	[Spermidine] (mM)	$s_{oc}$	$s_{20,w}$	$s_{20,w}$
3.0	–	–	11.33 ± 0.11	22.1	20.0
0.2	–	–	19.24 ± 0.05	21.0	19.7
0.07	–	–	19.88 ± 0.16	20.6	ND
0.03	–	–	19.67 ± 0.09	19.8	19.3
0.01	–	–	18.68 ± 0.03	18.7	18.8
0.1	0.01	–	20.45 ± 0.08	ND	ND
0.01	0.01	–	20.95 ± 0.09	ND	ND
0.001	0.01	–	21.22 ± 0.10	ND	ND
0.001	0.1	–	20.18 ± 0.02	ND	ND
0.02	0.01	3.5	20.89 ± 0.05	ND	ND

The sedimentation coefficients for open circular DNA are given in Svedberg units without ( $s_{oc}$ ) and with ( $s_{20,w}$ ) correction to standard conditions. The correction of  $s$  to standard conditions was done as described previously (Cohen & Eisenberg, 1968; Rinehart & Hearst, 1972). The calculated values of  $s_{20,w}$  are shown for the chain with the bead diameter,  $a$ , equal 3.18 nm (Hagerman & Zimm, 1981). Both measured and calculated values of  $s_{20,w}$  are rather sensitive to the values of the DNA buoyant density,  $(\partial\rho/\partial C)_u$  employed for correction. Therefore, throughout the paper we compare the values of the relative sedimentation coefficient,  $s/s_{oc}$ , which are independent of the correction procedure. The errors indicate standard deviations. ND, not determined.

conformations, the method which must be used in such an approach. One can now choose a model of the DNA double helix and calculate a measurable property such as the sedimentation coefficient or the radius of gyration of supercoiled DNA from the simulated conformational distribution. A comparison of the calculated and measured data shows the degree of agreement between the simulated conformations and the actual ones, and allows specification of the difference in conformational terms. Here, we performed an extensive study of supercoiled DNA conformations by comparing the measured and computed sedimentation coefficients of these molecules. In the accompanying paper (Rybenkov *et al.*, 1997a), we describe a dual theoretical and experimental analysis of supercoiling based on measures of catenation probability. In contrast to the sedimentation coefficient, which depends primarily on the overall size and the shape of the molecule, the catenation probability reflects more local features of superhelix conformations, and therefore these two methods complement each other.

Here, we studied the sedimentation coefficient of supercoiled DNA as a function of superhelical density,  $\sigma$ , in solutions containing 0.01 M to 3.0 M NaCl. The same dependencies of  $s$  on  $\sigma$  and on NaCl concentration were then computed and compared with the experimental data. We used 7 kb molecules rather than the smaller ones often used previously, because they are branched and therefore have more of the essential features of supercoiled DNA. We found a good agreement between the experimental and theoretical results. This allowed us to interpret in conformational terms the observed changes in sedimentation coefficient. We also measured the  $s$  values over a range of MgCl<sub>2</sub> and spermidine concentrations, including those which were reported to promote the collapse of interwound superhelix. However, we found no indication of such a collapse under any conditions even remotely approximating physiological ones.

The results of the accompanying paper reinforce and extend these conclusions, and we defer to the latter a general discussion of the results.

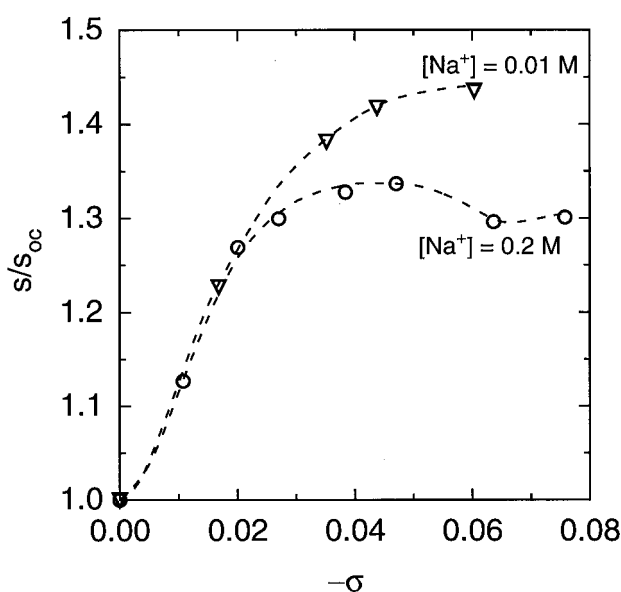
## Results and Discussion

### Sedimentation of supercoiled DNA in NaCl solutions

For each particular ionic condition and  $\sigma$  value we measured the sedimentation coefficient at several DNA concentrations and then extrapolated the value to a zero DNA concentration. The values of the DNA sedimentation coefficient reflect both the DNA conformations in a particular solvent and the physical-chemical properties of the solution, such as solvent viscosity and the DNA partial specific volume. The values of the DNA partial specific volume in NaCl and CsCl solutions have been measured previously (Cohen & Eisenberg, 1968). However, no such accurate data are available for MgCl<sub>2</sub> or spermidine-containing solutions. Therefore, we chose to monitor the relative sedimentation coefficient,  $s/s_{oc}$ , the ratio of sedimentation coefficients of supercoiled and open circular DNA, so that the solution physical-chemical properties would cancel.

The extrapolated values of  $s$  for the open circular DNA in all ionic conditions studied are shown in Table 1. For NaCl solutions, the values of sedimentation coefficient corrected to standard conditions,  $s_{20,w}$ , are also shown. The change of  $s_{20,w}$  reflects the change in conformations of open circular DNA with salt concentration and similar changes of  $s_{20,w}$  have been observed for linear DNA (Rinehart & Hearst, 1972). The standard deviation in the  $s$  values averages to only 0.5% of the mean, which is much lower than the change in  $s_{oc}$  with ionic conditions that we measured.

We measured the sedimentation coefficients of 7 kb supercoiled DNA as a function of superhelical density,  $\sigma$ , in 0.2 M NaCl and in 0.01 M NaCl

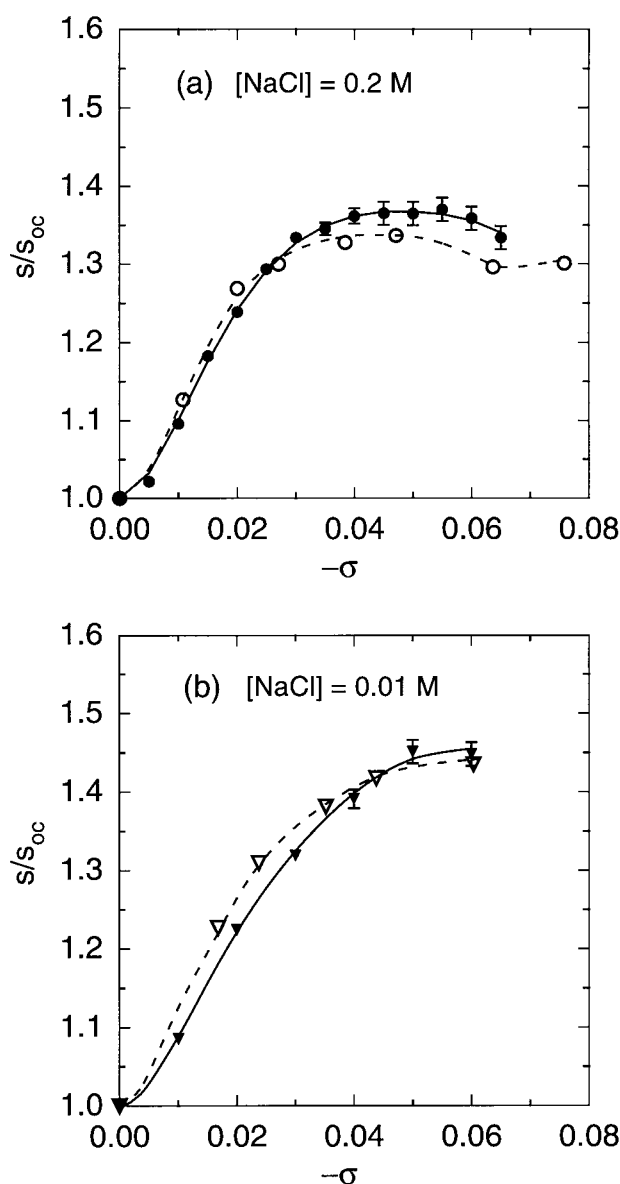


**Figure 1.** The measured relative sedimentation coefficient of DNA as a function of superhelix density and salt concentration. The measurements were for 7.0 kb DNA in 0.2 M NaCl ( $\circ$ ) and 0.01 M NaCl ( $\nabla$ ). The relative sedimentation coefficient,  $s/s_{oc}$ , is the value of the sedimentation coefficient normalized to that of open circular DNA for the corresponding conditions.  $\sigma$ , superhelix density.

(Figure 1) and found that the conformations of supercoiled DNA are different for these two ionic conditions. Supercoiling compacts DNA and therefore lowers its frictional coefficient and increases  $s/s_{oc}$  for both ionic conditions, but this effect is greater at the low salt concentration. Moreover, the compaction reaches a maximum at  $\sigma$  of  $-0.04$  to  $-0.05$  for the high salt concentration, but no maximum is observed at the low concentration of NaCl. The dependence of  $s/s_{oc}$  on  $\sigma$  that we measured in 0.2 M NaCl is in good agreement with the results obtained previously for DNA of a similar size in 3 M CsCl (Wang, 1974).

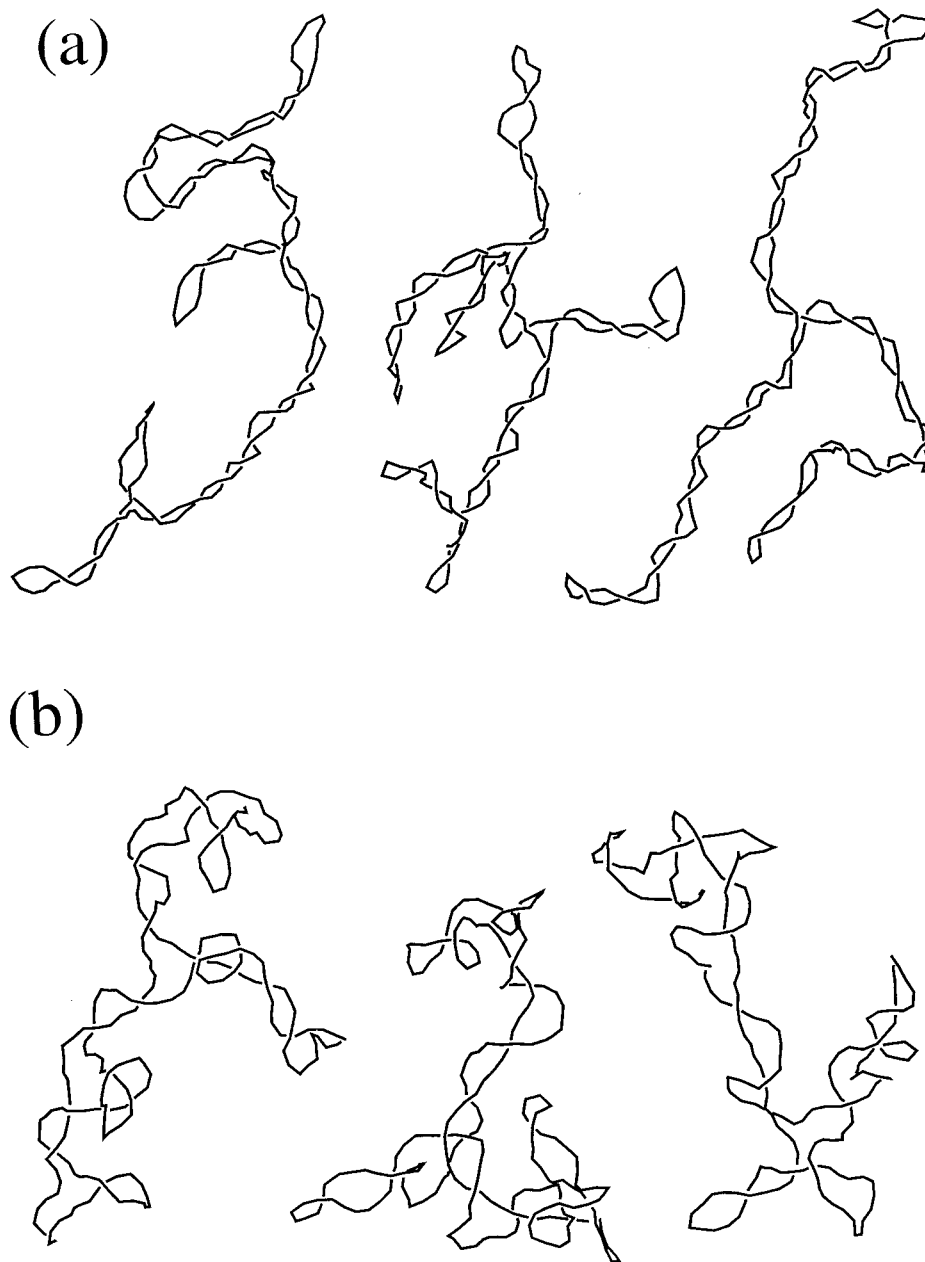
We calculated the dependence of  $s/s_{oc}$  on  $\sigma$  for both 0.2 M NaCl and 0.01 M NaCl. The calculations were made for random samples of DNA conformations obtained by Monte Carlo simulation, a computational method that generates the equilibrium conformational distribution for a model DNA chain. In the range of ionic conditions used in this study, the only parameter of the model that varied as a function of ionic conditions was the DNA effective diameter. This parameter specifies the electrostatic intersegment interaction and its values have been accurately defined (see Methods for details).

Figure 2 shows the results of the calculations together with experimental data of Figure 1 demonstrating good agreement, within 5%, between the experimental data and the calculations. Throughout this and the accompanying paper, the measured data are represented by open symbols



**Figure 2.** Comparison of the measured and calculated relative sedimentation coefficients for 7 kb DNA. The data correspond to NaCl concentrations of 0.2 M (a) and 0.01 M (b). Throughout this and the accompanying paper, measured data are shown by open symbols and calculated data are depicted with filled symbols. The error bars for simulation data correspond to one standard deviation and are shown only when larger than the symbol size.

and the calculated data by filled symbols. The differences between calculated and measured values are greater than the error of the methods, but are significantly less than the changes of  $s/s_{oc}$  with NaCl concentration. The calculated values reproduce the shapes of the curves even to the finding of a maximum of  $s/s_{oc}$  at high but not low salt concentration. This agreement is especially gratifying because there were no adjustable parameters in the calculations. This convergence of values gives us confidence that the picture of supercoiled DNA



**Figure 3.** Typical simulated conformations of supercoiled DNA in solution containing 0.2 M NaCl (a) and 0.01 M NaCl (b). The conformations of the model chains correspond to DNA 7 kb in length and a  $\sigma$  value of  $-0.05$ .

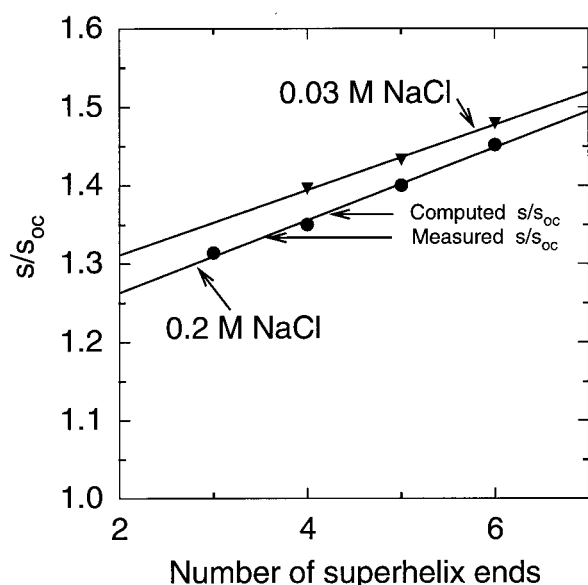
conformations we obtained from computer simulations faithfully reflects the actual conformational properties of supercoiled DNA in solution, and allows us to make a detailed interpretation of the experimental results in conformational terms.

### Structural interpretation

The maximum of  $s/s_{oc}$  near a  $\sigma$  value of  $-0.05$  at high ionic strength (Figure 2) has previously been observed (Wang, 1969,1974; Upholt *et al.*, 1971). The interpretation initially suggested was that the superhelix changed from a toroidal form to the more extended plectonemic form in this range of  $\sigma$ . Our simulation results support a different in-

terpretation of the maximum: they show that the plectonemic form is the only regular conformation of supercoiled DNA. This superhelix is, however, often branched (Figure 3), which compacts the molecules and therefore increases the value of  $s$ . Because branching frequency decreases for higher supercoiling (Vologodskii *et al.*, 1992),  $s$  should also decrease, resulting in a maximum in  $s$  caused by a maximum in branching.

Neither the experimental data nor the simulations show a maximum of  $s$  in 0.01 M NaCl (Figure 2b). The simulated conformations are more open and loose at this ion concentration (Figure 3b). They are so irregular at all values of  $\sigma$  that even a direct inspection of a conformation does not lead to an un-



**Figure 4.** The calculated relative sedimentation coefficient of supercoiled DNA as a function of superhelix branch number. The set of simulated conformations was divided into subsets according to the number of superhelix ends of the simulated conformations, and the values of  $s/s_{oc}$  were calculated separately for the each subset. The number of branches is approximately two less than the number of ends. The calculations corresponded to supercoiled DNA 7 kb in length,  $\sigma = -0.05$ , in 0.2 M NaCl (●) or 0.03 M NaCl (▼). For 0.2 M NaCl, the average value of  $s/s_{oc}$  for all simulated conformations (Computed  $s/s_{oc}$ ) and the measured value (Measured  $s/s_{oc}$ ) of  $s/s_{oc}$  are also shown.

ambiguous definition of the number of branches. Thus, it is not surprising that  $s$  monotonically changes with  $\sigma$  (Figure 2b).

Among the parameters describing specific features of supercoiled DNA, we were particularly interested in branching frequency and in the average superhelix diameter. We developed a special computational experiment to determine the dependence of the calculated values of  $s$  on these parameters. We separated simulated conformations of supercoiled DNA ( $\sigma = -0.05$ ) according to the number of the superhelix branches and then calculated the average values of  $s/s_{oc}$  as a function of the branch number for both 0.2 M and 0.03 M NaCl solutions (Figure 4). For both salt concentrations, we found that the sedimentation coefficient is proportional to the number of branches, which strongly affect the average size of supercoiled molecules. However, we did not detect an effect of the superhelix diameter on the value of  $s$ . The value of the average superhelix diameter increases 30% when NaCl concentration is reduced from 0.2 M to 0.03 M (Table 2). This increase would be predicted to cause a decrease in  $s$  (see equation 4 in Methods). Instead we found a small increase in  $s/s_{oc}$  at the lower salt concentration (Figure 4) which

**Table 2.** Variation of DNA effective diameter and superhelix diameter ( $\sigma = -0.05$ ) as a function of NaCl concentration

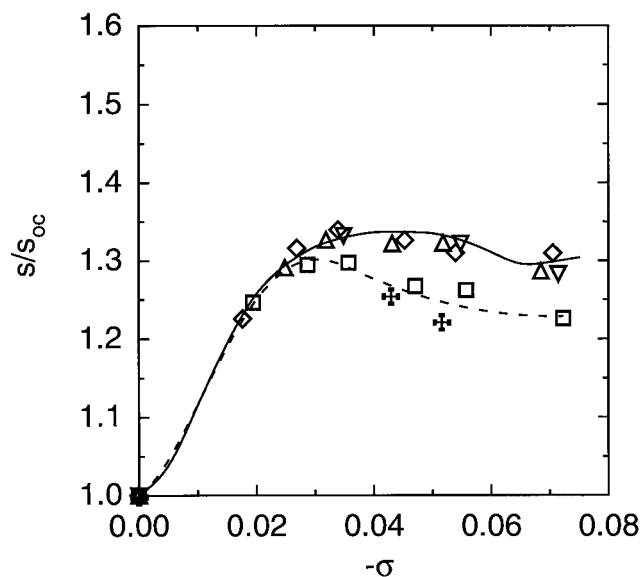
NaCl concentration (M)	DNA effective diameter (nm)	Superhelix diameter (nm)
0.01	15	17
0.02	11	14
0.03	9	13
0.2	5	10
1.0	3	9.5

The values of DNA effective diameter used in the simulations are shown (Stigter, 1977; Brian *et al.*, 1981; Rybenkov *et al.*, 1993; Shaw & Wang, 1993). The superhelix diameter,  $d_{sh}$ , is defined as the diameter of an idealized regular superhelix which has the same DNA contour length,  $L$ , superhelix pitch angle,  $\alpha$ , number of superhelix ends,  $n_e$ , and  $Wr$  as the average values of irregular superhelices (Boles *et al.*, 1990). We calculated superhelix diameter using:

$$d_{sh} = \frac{2L}{\pi(n_e + 4(-Wr)/\sin(2\alpha))}$$

where  $Wr$ ,  $\alpha$ , and  $n_e$  were the average values obtained from computer simulations.

can be explained by small changes in the global size of open circular and supercoiled DNA. Thus, the value of  $s/s_{oc}$  depends primarily on the global size and shape of supercoiled molecules and is much less sensitive, if at all, to the superhelix diameter. Figure 4 also shows that a small difference



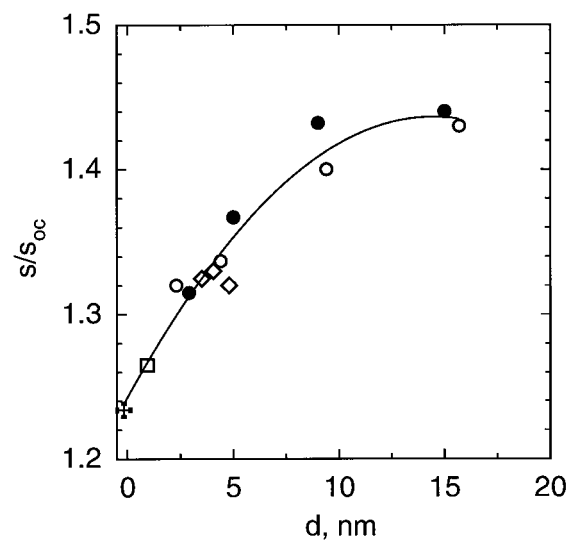
**Figure 5.** The measured relative sedimentation coefficients of 7 kb DNA in solutions containing  $MgCl_2$ . The open symbols correspond to solutions containing 10 mM  $MgCl_2$  and different concentrations of NaCl: 1 mM (▼), 10 mM (◇), and 100 mM (△). The continuous line corresponds to a solution containing 0.2 M NaCl only (reproduced from Figure 1). The broken line corresponds to the data measured for a solution containing 100 mM  $MgCl_2$  plus 1 mM NaCl (□) or 3.5 mM spermidine plus 10 mM sodium phosphate and 10 mM  $MgCl_2$  (⊗).

in branching of actual and simulated conformations could be responsible for the discrepancy between measured and simulated values of  $s$ .

### Sedimentation of supercoiled DNA in solutions containing di- and trivalent cations

It is well known that many di- and trivalent cations affect DNA conformations much more strongly than monovalent ions. We studied first the effect of a moderate concentration of  $\text{MgCl}_2$  (10 mM) on the sedimentation of supercoiled DNA. We found that the dependence of  $s$  on  $\sigma$  in 10 mM  $\text{MgCl}_2$  is very similar to that in 0.2 M NaCl (Figure 5). Addition of 0.01 M or 0.1 M NaCl to a solution of 10 mM  $\text{MgCl}_2$  did not change this dependence of  $s$  on  $\sigma$ . Thus, the presence of 10 mM  $\text{MgCl}_2$  eliminated the change of supercoiled DNA conformations between 0.01 M and 0.2 M NaCl. We conclude that within the physiological range of salt conditions, supercoiled DNA adopts essentially the same set of conformations.

We next studied the sedimentation of supercoiled DNA in extreme ionic conditions, in which we expected some attraction between double helix segments. We observed that 100 mM  $\text{MgCl}_2$  lowered values of  $s/s_{oc}$  for supercoiled DNA (Figure 5). We found a very similar dependence of  $s/s_{oc}$  on  $\sigma$  in the presence of 3.5 mM spermidine, a concentration that results in aggregation of approximately 50% of the DNA (Krasnow & Cozzarelli, 1982). The values of  $s/s_{oc}$  for the non-aggregated fraction of DNA are shown in Figure 5. A lower concentration of spermidine, 1.5 mM, did not cause as significant a reduction in the value of  $s/s_{oc}$  as did 3.5 mM spermidine (data not shown). The change of the sedimentation rate under these extreme ionic conditions can be explained by the elimination of superhelix branching. As shown in Figure 4, an unbranched superhelical DNA would have  $s/s_{oc}$  of 1.27, similar to the value obtained. One interpretation of the reduction in branching is that the superhelix has collapsed. Indeed, Dubochet and co-workers observed by cryo EM a significant reduction of superhelix branching for collapsed conformations of the superhelix (Adrian *et al.*, 1990; Bednar *et al.*, 1994). In the accompanying paper, though, we show that the superhelix has not collapsed under even these extreme ionic conditions. This conclusion is supported by the data of Ma & Bloomfield (1994) who found no indications of DNA condensation at any concentrations of  $\text{MgCl}_2$ . Unfortunately, we cannot use computer simulations to determine the superhelix conformations under these conditions, which limits our interpretation of the corresponding data. The hard core approximation of the electrostatic interaction used in the simulations can fail for ionic conditions under which DNA segments attract one another as is the case at 100 mM  $\text{MgCl}_2$  and at 3.5 mM spermidine (Shaw & Wang, 1993; Rybenkov *et al.*, 1997).



**Figure 6.** The measured and calculated relative sedimentation coefficients of supercoiled DNA as a function of the DNA effective diameter,  $d$ . The DNA had a  $\sigma$  of  $-0.05$ . The measured values are for solutions containing different amounts of NaCl ( $\circ$ ), solutions containing 10 mM  $\text{MgCl}_2$  and different concentrations of NaCl ( $\diamond$ ), 100 mM  $\text{MgCl}_2$  plus 1 mM NaCl ( $\square$ ), and 3.5 mM spermidine plus 10 mM sodium phosphate and 10 mM  $\text{MgCl}_2$  ( $\ast$ ). Calculated values are shown with the filled circles ( $\bullet$ ).

### Electrostatic intersegment interaction defines the dependence of supercoiled DNA conformations on ionic conditions

Our results show that conformations of supercoiled DNA depend strongly on ionic conditions. This was predicted by computer simulations (Klenin *et al.*, 1991; Vologodskii *et al.*, 1992; Vologodskii & Cozzarelli, 1994) and by theoretical analysis (Hunt & Hearst, 1991) and was also supported by cryo EM (Adrian *et al.*, 1990; Bednar *et al.*, 1994). This dependence results primarily from the electrostatic repulsion of DNA segments in the dense conformation of the interwound superhelix. We specify the value of the intersegment electrostatic repulsion in terms of DNA effective diameter,  $d$  (Stigter, 1977); a parameter whose value changes from 3 nm for 1 M  $\text{Na}^+$  to 15 nm for 0.01 M  $\text{Na}^+$  (Stigter, 1977; Brian *et al.*, 1981; Yarmola *et al.*, 1985; Rybenkov *et al.*, 1993; Shaw & Wang, 1993). These changes cause significant alterations in the conformations (Figure 3) and the sedimentation (see Figure 1) of supercoiled DNA.

We recently measured the values of  $d$  for solutions containing mixtures of sodium, magnesium and spermidine ions (Rybenkov *et al.*, 1997b). This allowed us to compare the calculated and measured results for all ionic conditions studied. The experimental and calculated values of  $s$  vary only slightly near a  $\sigma$  of  $-0.05$  (Figure 2), and thus we chose  $s/s_{oc}$  at this  $\sigma$  as a characteristic value for particular ionic conditions. Both the measured and

simulated values of  $s/s_{oc}$  showed, within error scatter, monotonic dependence on  $d$  (Figure 6). Therefore, we conclude that the DNA effective diameter adequately specifies the conformational properties of supercoiled DNA not only for NaCl solutions but also for solutions with di- and trivalent ions. This issue is discussed in greater detail in the accompanying paper (Rybenkov *et al.*, 1997a).

## Methods

### DNA samples

The 7.02 kb plasmid, pAB4 (Wasserman *et al.*, 1988) was purified by the Triton lysis method (Ausubel *et al.*, 1989). To vary the superhelicity of the plasmid, the DNA was relaxed by wheat germ topoisomerase I (Dyran *et al.*, 1981) in the presence of different amounts of ethidium bromide. It was then extracted successively with phenol, phenol:chloroform and chloroform, precipitated with ethanol, resuspended in TE and filtered through a Sepharose CL-4B column. Seven different samples of closed circular DNA were prepared and their superhelical density was measured by band counting (Keller, 1975) relative to a sample relaxed in 0.1 M NaCl. To determine the superhelical densities of the samples at the ionic conditions of interest, we took into account the change in DNA helical repeat with temperature and ion concentration. The temperature coefficient,  $\Delta\sigma/\Delta T$ , has been shown to be  $3.1 \times 10^{-4}$  degree $^{-1}$  for all ionic conditions studied (Wang, 1969; Upholt *et al.*, 1971; Depew & Wang, 1975; Dugué, 1993). Variation of the superhelical density with NaCl and MgCl<sub>2</sub> concentration is also accurately known (Anderson & Bauer, 1978; Rybenkov *et al.*, 1997b). We used the value of  $\Delta\sigma/\Delta \lg[\text{Na}^+] = -2.5 \times 10^{-3}$  found for the 0.01 to 0.2 M NaCl solutions (Rybenkov *et al.*, 1997). For all samples and ionic conditions studied, the value of  $\sigma$  was determined with an accuracy of at least  $\pm 0.001$ . The only exception to this is the value in 3 M NaCl which was estimated by extrapolation and therefore could be in error by as much as  $\pm 0.005$ .

The extent of DNA aggregation by spermidine was determined by centrifugation (Krasnow & Cozzarelli, 1982). Singly nicked circular DNA was prepared by limited digestion by DNase I in the presence of ethidium bromide (Barzilai, 1973).

The nucleotide sequence of pAB4 DNA contains no long regions with a high AT-content, alternating purine-pyrimidine stretches, or palindromic sequences which could form open regions, Z form segments, and cruciforms, respectively, under low torsional stress. We also used a statistical-mechanical analysis (Anshelevich *et al.*, 1988) with the same objectives, which showed the absence of stable non-canonical structures at any  $\sigma$  between 0 and  $-0.06$ .

### Experimental measurement of sedimentation coefficients

The analytical ultracentrifuge Optima XL-A (Beckman) was used to measure the sedimentation coefficient,  $s$ . For each sample we measured the value of  $s$  for four to eight DNA concentrations in the range of 10 to 60  $\mu\text{g}/\text{ml}$  and extrapolated the data to a zero DNA concentration. The extrapolated sedimentation coefficients were obtained with a precision of 0.5%. All measurements were done at 20°C. The extrapolated values of  $s$  for open circular

DNA at all ionic conditions studied are shown in Table 1, together with their standard deviations. For MgCl<sub>2</sub>-containing solutions, these values are not corrected to standard conditions,  $s_{20,w}$ , because we lack accurate data on the corresponding values of the DNA partial specific volume. However, this correction is unnecessary because we report exclusively the relative sedimentation coefficient for supercoiled DNA, i.e. the ratio of the sedimentation coefficient of supercoiled DNA to that of open circular DNA.

Each DNA sample contained a mixture of topoisomers with an average value of the superhelix density,  $\langle\sigma\rangle$ . As a result, the measured value of  $s$ ,  $\langle s \rangle$  was slightly different from the value of  $s$  at the center of the distribution,  $s(\langle\sigma\rangle)$ , which was the calculated value. This difference, however, was small. The distribution of topoisomers can be described by a Gaussian with the average value  $\langle\sigma\rangle$  and the variance  $\langle(\delta\sigma)^2\rangle$ . Using this we can approximate  $\langle s \rangle$  as:

$$\langle s \rangle = s(\langle\sigma\rangle) + \frac{1}{2} \frac{d^2s(\sigma)}{d\sigma^2} \langle(\delta\sigma)^2\rangle.$$

The value of  $\langle(\delta\sigma)^2\rangle$  was equal to  $(1.2 \pm 0.3) \times 10^{-5}$  for our samples, and

$$\frac{d^2s(\sigma)}{d\sigma^2}$$

did not exceed  $2 \times 10^4$  for all experimental points. Thus  $|\langle s \rangle - s(\langle\sigma\rangle)|$  was always less than 0.2 S.

### Calculations of sedimentation coefficient

To calculate the value of  $s$  we used the usual replacement of flexible DNA molecules by an ensemble of rigid, randomly selected conformations. (For a discussion of this approximation, see Zimm, 1980.) To use this approach we first simulated the sets of conformations of supercoiled DNA for different conditions. These sets corresponded to the equilibrium conformational distributions of the model chains. In the second step, we calculated the average sedimentation coefficients for the sets. We next describe the model of the double helix used in the simulations.

### The DNA model

The model is a discrete analog of the worm-like chain which includes additional volume interactions. A DNA molecule composed of  $n$  Kuhn statistical lengths is modeled as a closed chain consisting of  $kn$  rigid segments that are cylinders of equal length. The elastic energy of the chain,  $E_b$  is computed as:

$$E_b = RT\alpha \sum_{i=1}^{kn} \theta_i^2 \quad (1)$$

where the summation extends over all the joints between the elementary segments,  $R$  is the gas constant,  $T$  is the absolute temperature,  $\theta_i$  is the angular displacement of segment  $i$  relative to segment  $i-1$ , and  $\alpha$  is the bending rigidity constant. The bending constant  $\alpha$  is defined so that the Kuhn statistical length corresponds to  $k$  rigid segments (Frank-Kamenetskii *et al.*, 1985).

We showed before that the simulated properties of supercoiled conformations do not change, within the accuracy of the simulations, if  $k \geq 10$  (Vologodskii *et al.*, 1992). We used a  $k$  value of 10 in this work.

The model also takes into account the volume interactions between DNA segments. We incorporated these interactions into the model *via* the concept of the effective diameter,  $d$ , the actual diameter of the cylindrical segments of the model. Its value takes into account not only the physical diameter of DNA, but also the electrostatic repulsion between DNA segments. The effective diameter of DNA is defined as the diameter of an uncharged model chain that mimics the conformational properties of actual DNA. Its quantitative definition is based on the concept of the second coefficient of virial expansion (Stigter, 1977). A more accurate approximation of electrostatic interactions based on the Debye-Hückel potential yields similar results (Vologodskii & Cozzarelli, 1995).

The energy of torsional deformation is considered to be independent of bending deformation and proportional to the displacement of DNA twist from its equilibrium value,  $\Delta Tw$ . The torsional energy was calculated as (Hao & Olson, 1989):

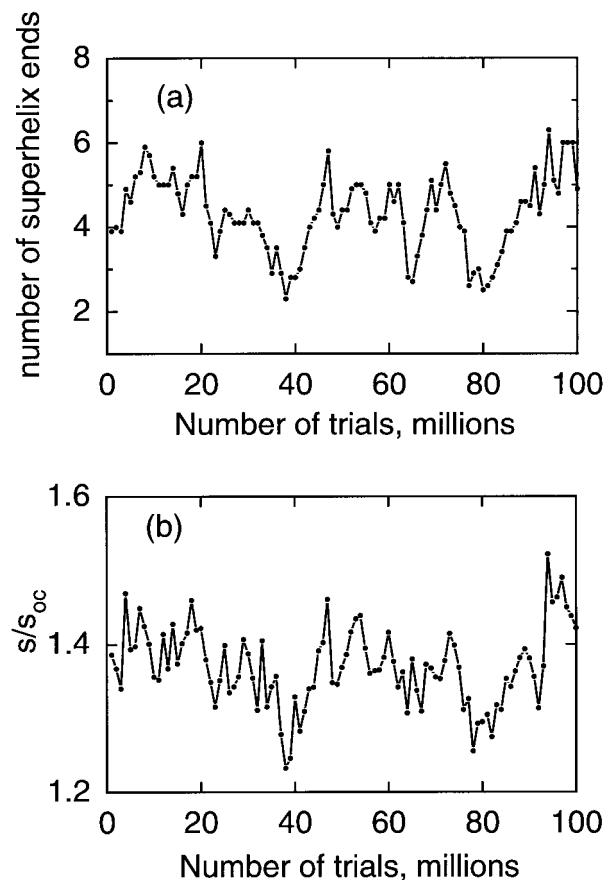
$$E_t = (2\pi^2 C/L)(\Delta Lk - Wr)^2 \quad (3)$$

where  $Wr$  is the writhe of the chain (White, 1989),  $\Delta Lk$  is the linking number difference of the supercoiled DNA,  $C$  is the torsional rigidity constant, and  $L$  is the DNA length. The value of  $\Delta Lk$  was a parameter in each simulation and  $Wr$  is calculated for each conformation.

Thus, there are three parameters of the model. All are well known from independent studies. The first parameter, the Kuhn statistical length, is about 100 nm for solutions containing more than 0.01 M monovalent ions or more than 1 mM multivalent ions (Hagerman, 1988). We use a value of  $3 \times 10^{-19}$  erg cm for  $C$  (Hagerman, 1988; Klenin *et al.*, 1989; Rybenkov *et al.*, 1997). This value of  $C$  gave better agreement between experimental and computed results than the lower value,  $2 \times 10^{-19}$  erg cm, accepted by some other workers (Gebe *et al.*, 1996; Heath *et al.*, 1996). For the lower value of  $C$ , the same value  $Wr$  is achieved at higher  $\Delta Lk$ . Since we consider torsional and bending distortions of DNA independent from each other, the conformations of supercoiled DNA are defined entirely by the value of  $Wr$ . Therefore, for the lower value of  $C$ , the calculated values of the sedimentation coefficient would correspond to the DNA with the higher degree of supercoiling. This increase in superhelical density that yielded the same value of  $Wr$  depended on ionic conditions and varied over the range of 10 to 15% of the value found for  $C$  equal  $3 \times 10^{-19}$  erg cm. The third parameter, DNA effective diameter,  $d$ , depends strongly on ionic conditions. For solutions of monovalent ions the values of  $d$  are known with accuracy from both experimental and theoretical methods (Stigter, 1977; Brian *et al.*, 1981; Rybenkov *et al.*, 1993; Shaw & Wang, 1993; Stigter & Dill, 1993). We used the values of  $d$  shown in Table 2.

### Generation of an equilibrium set of conformations

We used the Metropolis Monte Carlo procedure to simulate the equilibrium set of conformations of supercoiled molecules as previously detailed (Vologodskii *et al.*, 1992). In this procedure the set of system states is the result of successive deformations from an initial state. The deformations included two types of motions. The first type can be described as a crankshaft rotation (Klenin *et al.*, 1991). Here we modified this type of motion to increase the rate of sampling of different conformations of the interwound superhelix. A portion of the chain con-



**Figure 7.** Variation of the calculated number of superhelix ends (a) and relative sedimentation coefficient (b) during a simulation run. Each point corresponds to an average of over  $10^6$  trial moves. The data show that even for these most slowly changing features of simulated conformations, there was no notable correlation between successive conformations separated by more than  $10^7$  steps.

taining an arbitrary number of adjacent segments was rotated by an angle,  $\phi$ , around a line connecting the ends of the sub-chain. The value of  $\phi$  was uniformly distributed over a range  $(-\phi_0, \phi_0)$ . If the end-to-end distance of the sub-chain,  $R$ , exceeded the length of four chain segments, no rotation was made and the current conformation was counted as a trial one. The value of the  $\phi_0$  was chosen so that the probability of acceptance for a crankshaft move, for  $R < 4$ , was about 0.5. By excluding rotations for large values of  $R$  (which are strongly restricted in an interwound superhelix) we could increase by a factor of 10 the average amplitude of rotation for small values of  $R$ . Some of these rotations correspond to a bending of an interwound superhelix and thus are important in obtaining an equilibrium sampling of global superhelix conformations. This type of move was  $R$ -independent if  $|\sigma| \leq 0.02$ , conditions under which DNA conformations are loose, and only scattered elements of an interwound superhelix are present.

The second type of motion, reptation, was introduced into the simulations to increase the rate of change of the superhelix branch number (Vologodskii *et al.*, 1992). There were about 20 attempts of rotation per one at-

tempt of reptation for moderate to highly supercoiled DNA. We used only  $R$ -independent crankshaft rotation for low  $|\sigma|$ .

We tested each trial conformation for overlap of non-adjacent segments. A trial conformation was rejected if the distance between two non-adjacent segments was smaller than  $d$ . The starting conformations were unknotted. However, this did not guarantee that the chain remained unknotted, because segments of the chain were allowed to cross during trial moves. Therefore we checked the topology of the trial conformation by evaluating the Alexander polynomial,  $\Delta(t)$ , at  $t = -1$  (Frank-Kamenetskii & Vologodskii, 1981). If a trial conformation was knotted, it was rejected.

If a trial conformation passed the tests of absence of chain overlap and knotting, its elastic energy was calculated as a sum of  $E_b$  and  $E_t$  (equations 1 and 3). This required calculating the writhe of the trial conformation, which is most conveniently done by the method of Le Bret (1980). The probability of accepting a trial conformation was obtained by applying the classical rules of Metropolis *et al.* (1953).

The current conformation did not change after most steps of this algorithm for  $|\sigma| > 0.02$ , because  $R$  was greater than four for these steps. Although one must consider these steps as unsuccessful moves, they took very little computer time. Therefore we were able to use as many as  $4 \times 10^8$  steps for sampling highly supercoiled 7 kb DNA for each particular condition. Each run took about 100 hours of processor time on a Silicon Graphics Indigo2 workstation. There was no notable correlation between successive conformations separated by more than  $10^7$  steps. This is illustrated in Figure 7, in which the changes of the branch number and the sedimentation coefficient, the most slowly changing features of supercoiled DNA, are shown for a quarter of a simulation run.

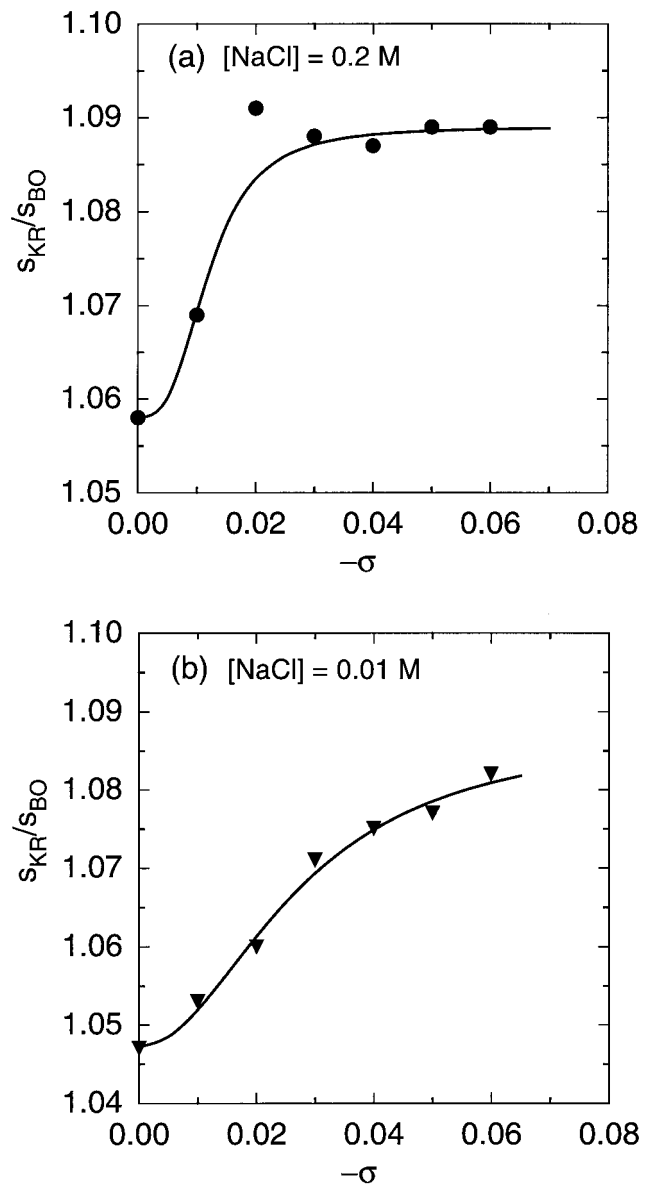
There are several reasons to conclude that the algorithm described provides equilibrium sampling of DNA conformations. We found that our algorithm gave the same results when starting from very different initial conformations. Our previous simulation results were confirmed by the experimental data (Vologodskii *et al.*, 1992; Vologodskii & Cozzarelli, 1994). Some of our earlier simulation results were also confirmed recently by Gebe *et al.* (1995) who used a slightly different DNA model and different moves in their Metropolis algorithm.

We used an algorithm previously described (Vologodskii *et al.*, 1992) to calculate the number of superhelix branches in a particular conformation.

### Calculation of sedimentation coefficient

To calculate  $s$  values for simulated conformations, we substituted for a chain consisting of  $kn$  straight cylindrical segments a chain consisting of  $3kn$  touching beads. The diameter of the beads can be varied by changing the segment length. Values close to 3 nm were used for the bead diameter to approximate the hydrodynamic properties of the double helix (Kovacic & van Holde, 1977; Hagerman & Zimm, 1981; Langowski *et al.*, 1994). We found that the ratio of  $s$  values for supercoiled and open circular DNA,  $s/s_{oc}$ , does not depend on bead diameter if it is between 2.5 and 3.33 nm. Thus, to reduce the number of beads in the chain, we used a diameter of 3.33 nm for most of the simulations.

We used the Kirkwood-Riseman approximation to calculate the sedimentation coefficient of each individual



**Figure 8.** Comparison of the sedimentation coefficients calculated using the Kirkwood-Riseman approximation,  $s_{KR}$ , with that obtained from a direct solution of the Burgers-Oseen problem,  $s_{BO}$ . The calculations were done for supercoiled DNA 7 kb in length. For each value of  $\sigma$  and a NaCl concentration of 0.2 M (a) or 0.01 M (b), we calculated the average values of  $s_{KR}/s_{BO}$  over three orientations of 25 randomly chosen conformations.

chain conformation,  $s_m$ , from the constructed equilibrium set (Bloomfield *et al.*, 1974):

$$s_m = \left( \frac{\partial \rho}{\partial C} \right)_\mu \frac{M}{N_A} \frac{1}{N} \frac{1}{3\pi\eta_0 a} \left( 1 + \frac{r}{N} \sum_{i=1}^N \sum_{j=1}^N r_{ij}^{-1} \right) \quad (4)$$

where  $N_A$  is Avogadro's number,  $M$  is the molecular weight of DNA,  $(\partial \rho / \partial C)_\mu$  is the DNA buoyant density,  $N$  is the number of the beads in the chain,  $\eta_0$  is the viscosity of solution,  $a$  is the diameter of the beads, and  $r_{ij}$  is the distance between beads  $i$  and  $j$ . We then calculated

the average value of  $s$  for the conformational set. It is important that the only term in this expression that depends on DNA conformation is  $r_{ij}^{-1}$ ; all other terms cancel when we calculate  $s/s_{oc}$ .

Although we avoided the pre-averaging of the hydrodynamic interaction between chain segments through different chain conformations, equation (4) still gives only an approximate solution of the Burgers-Oseen problem. To evaluate the quality of the approximation, we compared, for a part of the conformations, the results of the Kirkwood-Riseman approximation with a direct solution of the Burgers-Oseen problem as proposed by Zimm (1980).

For any particular conformation of a chain described by the position of  $N$  beads, the Burgers-Oseen problem is reduced to a system of  $(3N + 4)$  linear equations. The coefficients of these linear equations are composed mainly from the tensor of hydrodynamic interaction. We used the Rotne-Prager approximation (Rotne & Prager, 1969) for the tensor:

$$\mathbf{T}_{ij} = \frac{1}{8\pi\eta_0 r_{ij}} \left[ \left( \mathbf{I} + \frac{\mathbf{r}_{ij}\mathbf{r}_{ij}}{r_{ij}^2} \right) + \frac{a^2}{2r_{ij}^2} \left( \frac{1}{3}\mathbf{I} - \frac{\mathbf{r}_{ij}\mathbf{r}_{ij}}{r_{ij}^2} \right) \right] \quad (5)$$

where  $\mathbf{r}_{ij}$  specifies the interparticle distances,  $\mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j$ , and  $\mathbf{I}$  is the unit tensor. The first term in the sum, which is proportional to  $1/r_{ij}$ , is the commonly used Oseen tensor, and the second term accounts for finite bead size.

The value of  $N$  was equal to 708 in our case (when the diameter of the beads was equal to 3.33 nm) and it took 20 minutes to solve the system for one orientation of one particular conformation of the chain on the Silicon Graphics Indigo2 workstation.

We used both the Kirkwood-Riseman approximation and the exact solution to calculate the sedimentation coefficient for three different orientations of 25 uncorrelated conformations for each set of superhelix density and ionic conditions. We found that the Kirkwood-Riseman equation overestimates the sedimentation coefficient by 5 to 10% for our model chain, depending on DNA superhelix density (Figure 8). Using these data we obtained a correction coefficient for the Kirkwood-Riseman approximation. The value of the coefficient,  $K_{corr}(\sigma)$ , changes from 1.0 for  $\sigma$  equal 0 to approximately 0.96 for  $\sigma$  equal  $-0.06$  (Figure 8). Thus, to calculate the sedimentation coefficient for each  $\sigma$  and salt concentration, the average  $s/s_{oc}$  obtained by the Kirkwood-Riseman approximation for a large array of simulated conformations was multiplied by  $K_{corr}(\sigma)$ .

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