Flow of Structural Information between Four DNA Conformational Levels†

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Received October 25, 1995⊗

ABSTRACT: Closed-circular supercoiled DNA molecules have been shown to form a cholesteric assembly within bacteria as well as *in vitro* under physiological DNA and salt concentrations. Circular dichroism and X-ray scattering studies indicate that the macroscopic structural properties of the chiral mesophase are directly and uniquely dictated by the supercoiling parameters of the constituent molecules. Specifically, we find that the pitch of the DNA cholesteric phase derived from supercoiled DNA is determined by the superhelical density, which, in turn, is modulated by secondary conformational changes. A direct interrelationship among four DNA structural levels, namely, DNA sequence, secondary structural transitions, the tertiary superhelical conformation, and the quaternary, supramolecular organization is accordingly pointed out. Since secondary conformational changes are both sequence and environment dependent, alterations of cellular conditions may effectively modulate the properties of the packed DNA organization, through their effects on secondary structural transitions and hence on the superhelical parameters. On the basis of these results we suggest that liquid crystallinity represents an effectively regulated packaging mode of plectonemic, nucleosome-free DNA molecules in living systems.

It has recently been reported that supercoiled DNA molecules spontaneously form a cholesteric liquid-crystalline phase at physiological DNA and salt concentrations (Reich et al., 1994a,b). X-ray scattering experiments conducted on intact bacteria indicated that plasmids segregate within bacteria into condensed clusters characterized by a long-range lateral order (Reich et al., 1994a). The significance of these observations lies in the fact that several families of bacterial plasmids replicate into a very large copy number which amounts to a total amount of DNA that may exceed the amount of the bacterial genomic DNA. Since the extent of intramolecular DNA compaction associated with a plectonemic conformation is negligible (Boles et al., 1990), such a copy number necessarily imposes severe organizational demands upon the host. Thus, the observed plasmid assembly, obtained exclusively through intermolecular packaging processes, represents an efficient storage mode for nucleosome-free DNA molecules.

It is becoming increasingly evident that DNA microheterogeneity, that is, the formation of short non-B-DNA segments which are interspersed within the B-type double helix, may play an important role in promoting and regulating DNA condensation processes (Reich et al., 1991). Specifically, structurally-modified DNA clusters such as the left-handed Z motif were found to exhibit altered charge densities and, as such, to facilitate a close approach of the double helices (Ma et al., 1995). Moreover, conformational perturbations which occur at the junction sites between native B-form and structurally-modified segments were shown to increase the elastic response of DNA molecules and hence to promote their collapse (Reich et al., 1991).

The modified charge densities and increased flexibility associated with DNA structural microheterogeneity are proposed to promote and regulate predominantly *intramolecular* packaging of nucleic acids, where the occurrence of substantial folding and kinking is required. The relatively rigid rod-like conformation of the plasmid molecules specifically enhances, however, the formation of a cholesteric phase through *intermolecular* packaging pathways. What, then, are the factors taht control the packaging of closed circular supercoiled DNA molecule and determine the structural properties of the resulting assemblies? We have recently shown that the properties of cholesteric aggregates derived from plasmid molecules are dictated by the density and handedness of the molecular superhelicity. It has been argued that the strict correlation between the molecular parameters on one hand, and the supramolecular features exhibited by the plasmid-derived mesophase on the other, is a unique trait of plectonemic DNA molecules; the supramolecular features of all hitherto studied lyotropic mesophases obtained from biopolymers depend predominantly on environmental parameters (Reich et al., 1994a,b).

Following these observations we sought to study supercoiling-mediated effects of localized secondary structural transitions upon the liquid-crystalline phases of superhelical DNA molecules. Toward this aim, alternating purine−pyrimidine segments capable of undergoing a B-to-Z transition were inserted into plasmids, and their effects upon supercoiling and packaging were investigated. A clear interrelationship among four DNA structural levels is detected: the supramolecular properties of the plasmid-derived cholesteric phases modulate—but are also determined by—the molecular supercoiling, which, in turn, is affected by secondary B-to-Z transitions whose extend is dictated by the primary sequence. The results indicate that localized supercoiling-promoted secondary structural transitions profoundly alter and control the properties of condensed mesophases derived from supercoiled DNA molecules.

† Supported by the Minerva Foundation, Germany.
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⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1996.

0006-2960/96/0435-2985$12.00/0

THEORETICAL BACKGROUND

Optical Properties of Cholesteric DNA Phases. DNA molecules possess an intrinsic tendency to form cholesteric liquid-crystalline phases once a critical nucleic acid concentration, which might be either macroscopic or local, has been reached (Keller & Bustamante, 1986; Livolant & Maestre, 1988; Yevdokimov et al., 1988; Strzelecka et al., 1988; Leforestier & Livolant, 1993). The cholesteric DNA phases exhibit characteristic optical properties, including strong birefringence and non-conservative ellipticities which can be monitored by circular dichroism (CD). The anomalous CD signals result from two general properties of the cholesteric phase: a long range chiral organization associated with the cholesteric twist, and an efficient delocalization of the light-induced excitation of the chromophores throughout the entire phase.

The sign of the non-conservative ellipticities reflects the sense of the supramolecular cholesteric twist: positive non-conservative CD signals result from a right-handed cholesteric twist, whereas negative signals correspond to a left-handed long-range chirality (Livolant & Maestre, 1988; Maestre & Reich, 1980). The magnitude of the non-conservative ellipticities exhibited by a cholesteric DNA organization is determined by several factors (Kim et al., 1986). These include the chromophore density within the chiral phase and the size of the chiral aggregates. An important additional factor is the cholesteric pitch: for a given size of the chiral aggregate, the magnitude of the ellipticities substantially increases as the pitch becomes larger. By using the CD technique it has recently been shown that, in contrast to hitherto characterized lyotropic phases of biopolymers, which show an indirect dependence of the cholesteric handedness and pitch upon the microscopic properties of the constituent molecules, the features of the mesophases derived from closed circular supercoiled DNA are determined by the molecular superhelical parameters (Reich et al., 1994a,b). Thus, the structural properties of the assembly directly reflect the conformational characteristics of the interwound molecules which form the mesophase.

Supercoiling and Z-DNA Motif. Native covalently-closed circular DNA molecules exhibit a negative, right-handed supercoiling. Being at a high-energy state (Vinograd et al., 1968; Ellison et al., 1985), superhelicity promotes processes that relax it, such as drug intercalation or a B-to-Z DNA transition. Such a transition is further enhanced by the synergistic effect of high ionic strength and dehydration (Zacharias et al., 1982). For long alternating purine−pyrimidine segments, a supercoiling-mediated process results in only a limited right- to left-handed transition since the supercoiling energy is used up as the transition proceeds (Stirdivant et al., 1982; Ellison et al., 1987; Albert et al., 1994). Thus, partial relaxation can be affected through either an equilibrium between relaxed forms (corresponding to a complete transition of the alternating purine−pyrimidine segment) and unrelaxed states or a transition into the left-handed motif that occurs within a limited part of the alternating purine−pyrimidine segment (Stirdivant et al., 1982). It is assumed that for long $(CG)_N$ segments, a fully cooperative transition and hence a rapid equilibrium between relaxed and nonrelaxed states occurs (Stirdivant et al., 1982). Yet, for $(TG)_N$ segments a partial relaxation may reflect a "true" segmental, non-cooperative transition (Albert et al., 1994).

Table 1: Recombinant Plasmids

plasmids	inserts	sites
BS32	$(CG)_{16}$	*Sma*I
BS64	$(CG)_8(TG)_6(CG)_4(TG)_6(CG)_8$	*Bam*HI
BS76	$(CG)_8(TG)_6(CG)_4(TG)_6(CG)_8$ and $(CG)_6$	*Bam*HI *Sma*I

EXPERIMENTAL PROCEDURES

Chemicals, Enzymes, and Oligonucleotides. Ethidium bromide, chloroquine, Tris base, ATP (equine muscle, disodium salt), and agarose (type II) were products of Sigma (St. Louis, MO). All DNA-modifying enzymes and restriction endonucleases were purchased from New England Biolabs (Beverly, MA). Oligonucleotides were synthesized on an 380B automated DNA synthesizer (Applied Biosystems).

Construction of Recombinant Plasmids. DNA molecules studied were BlueScript plasmid (BS, 2960 base pairs) and its recombinant derivatives (Table 1). BS32 was constructed by restricting BS with the single-site endonuclease *Sma*I followed by dephosphorylation and blunt-end ligation with the 32-bp alternating $(CG)_{16}$. BS64 was obtained by restricting BS with *Bam*HI, dephosphorylation, and ligation with $CGCGGATCC(CG)_8(TG)_6(CG)_4(TG)_6(CG)_8GGATCCG$, which has been annealed to its complementary strand and treated with *Bam*HI. BS76 was obtained by inserting the alternating $(CG)_{16}$ fragment into the *Sma*I site of BS64. Following transfection, the $(CG)_{16}$ segment sustained complete or partial deletions; the recombinant containing the largest alternating fragment [i.e., $(CG)_6$] was isolated.

Plasmid Preparation. Escherichia coli JM109 bacteria transfected with BS, BS32, and BS64 were grown on LB medium, whereas bacteria transfected with BS76 were grown on LB medium supplemented with 0.4 M NaCl. All media contained 50 μg of ampicillin/mL. Plasmids were isolated by standard methods (Sambrook et al., 1989). Linear forms of the plasmids were obtained by cutting with the single-site *Sca*I. A complete population of topoisomers of BS and BS64 was prepared by incubating the plasmids (3 μg) with 2 μL of rat liver topoisomerase I, in the presence of increasing amounts of ethidium bromide (0−8 μM) at 37 °C for 3 h (Bowater et al., 1992), followed by phenol−chloroform extraction and ethanol precipitation. Topoisomers covering the entire range of ΔLk were pooled together and used for two-dimensional gel electrophoresis.

DNA Packaging. A 10 μL amount of a solution containing 3.5 mg of DNA/mL was treated with the appropriate amounts of 5 M NaCl or 0.1 M $MgCl_2$ solutions to provide the final salt concentrations indicated in the figure legends. The samples were diluted with H_2O and brought to a volume of 1 mL with either 200 (20%) or 350 (35%) μL of EtOH. Addition of EtOH was always carried out last, 10 min prior to the spectroscopic measurements.

Circular Dichroism. CD spectra were recorded in a 1-cm light path cell on a Jasco J-500 spectropolarimeter equipped with a DP-500N data processor. Final DNA concentration in all samples: 35 μg/mL ($=5 \times 10^{-5}$ M, in base pairs).

Two-Dimensional Gel Electrophoresis. Topoisomers of BS and BS64 were electrophoresed on 1.2% agarose gels

(20 × 20 cm). Both dimensions were run at 4 °C, to eliminate local melting, for 22 h, at 110 V; the second dimension was run in the presence of 1.8 μL of chloroquine/mL (Bowater et al., 1992).

Electron Microscopy. Samples for shadowing electron microscopy were prepared by the Kleinschmidt method (Kleinschmidt et al., 1959). Aliquots of 100 μL of DNA (3.5 μg/mL) in 0.5 M ammonium acetate, 0.1 M Tris (at the pH indicated in the figure legend), and 2.5 mM EDTA were treated with 10 μL of cytochrome *C* (2.5 mg/mL) and spread on the surface of a solution of the above buffer diluted in a ratio of 1:20. The resulting monolayers were lifted onto parlodion-coated grids, washed, and blotted dry. Grids were rotary shadowed with platinum−palladium (80:20) and examined on an EM-400T.

Small-Angle X-ray Scattering. Condensed DNA phases were prepared by dissolving a known amount of EtOH-precipitated DNA in a 10 mM $MgCl_2$ solution, which was transferred into 1.5 mm quartz capillaries. X-ray measurements were conducted on a low-angle camera operating with Cu Kα radiation ($\lambda = 1.541$Å), monochromated with a Ni filter followed by a single Franks mirror. Scattering profiles were recorded with a linear position-sensitive detector; data acquisition time was 4 h at 19 °C.

RESULTS

Construction of Plasmids Containing Alternating Purine−Pyrimidine Segments. The DNA molecules used in this study were the BlueScript plasmid (BS, 2960 base pairs) and its recombinant derivatives. The longer insert, i.e., the sequence composed of 64 alternating purine−pyrimidine residues, was constructed in blocks of $(CG)_N$ and $(TG)_N$ in order to reduce the probability of deletions and cruciforms. Whereas BS32 and BS64 were readily obtained, attempts to construct a recombinant plasmid composed of both the 32 and 64 alternating purine−pyrimidine segments, using either the BS32 or the BS64 as parent cloning vectors, were unsuccessful. Following both pathways, the fragment containing the $(CG)_{16}$ segment was completely deleted. This problem was mitigated when bacteria transformed with BS64 to which the $(CG)_{16}$ fragment has been inserted at the *Sma*I site were grown on LB media supplemented with 0.3 M NaCl that affect an increase of the negative superhelical density [Higgins et al. (1988)]. Growth under such conditions reduced the extent of deletions and allowed the isolation of a construct, coined BS76, in which a $(CG)_6$ sequence [derived from the $(CG)_{16}$ segment] was stably integrated at the *Sma*I site.

Shadowing Electron Microscopy and Gel Electrophoresis Studies. Isolated and purified samples of BS, BS32, BS64, and BS76 were studied by shadowing electron microscopy (Figure 1). A clear trend of increased relaxation is observed as a function of the length of the potentially Z-forming segments. Whereas the BS molecules appear as highly supercoiled species, a gradual decrease of the superhelical density is exhibited by BS32 and BS64, and a nearly relaxed topology is exhibited by the BS76 plasmids. The correlation between the size of the alternating purine−pyrimidine segments and the gradually enhanced relaxation implies that under the conditions used to prepare the specimens for shadowing electron microscopy a complete transition of the alternating inserts into the Z form has been induced within all recombinant plasmids. Specifically, the topological differences observed between the various constructs suggest that a left-handed conformation is not limited to $(CG)_N$ sequences and is adopted by alternating $(TG)_N$ segments as well, as indeed previously demonstrated (Ellison et al., 1987; Albert et al., 1994). Moreover, the difference between the apparent topology of BS64 and BS76 (Figure 1, panels C and D) implies that both inserts in BS76 undergo a B-to-Z transition which is reflected by the relatively enhanced relaxation.

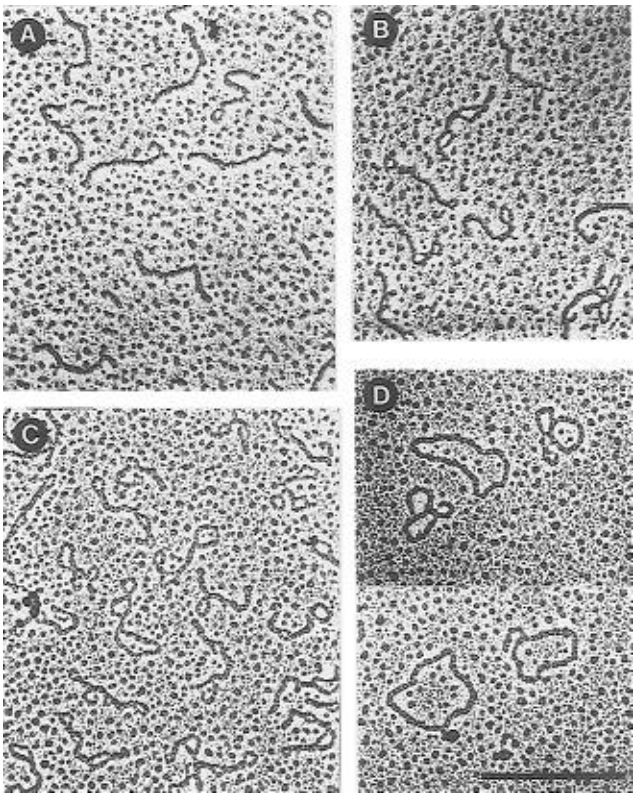

FIGURE 1: Electron microscopy of metal-shadowed samples of (A) BS, (B) BS32, (C) BS64, and (D) BS76. Spreading and shadowing procedures are detailed under Experimental Procedures. Scale bar, 0.5 μm.

The occurrence of a secondary conformational change within the recombinant plasmids is further indicated by the effects of such a transition on the electrophoretic mobility of topoisomers derived from BS and BS64 on a two-dimensional gel. As expected, two-dimensional gel electrophoresis of the topoisomers obtained from BS did not reveal any alterations in the twist, thus indicating the absence of supercoiling-dependent secondary structural transitions. A sharp structural transition with $\Delta Tw \approx -3$ is observed, however, for BS64 at topoisomer $\Delta Lk = -10$ (superhelical density of −0.035), corresponding to the formation of Z-DNA by a limited portion (≈16 base pairs) of the alternating purine−pyrimidine insert (Figure 2A). An additional reduction in twist, implying the induction of the Z motif in a larger portion of the insert, is exhibited by $\Delta Lk = -12$. The stepwise relaxation is assigned to the presence of alternating blocks of CG and TG within the insert: since the energy required for a B-to-Z transition of alternating TG residues is significantly higher than the energy needed for this process in CG base pairs (Peck & Wang, 1983; Anshelevich et al., 1988), a superhelical density that allows the formation of the Z motif in an alternating CG segment may not be sufficient to affect the process in the alternating

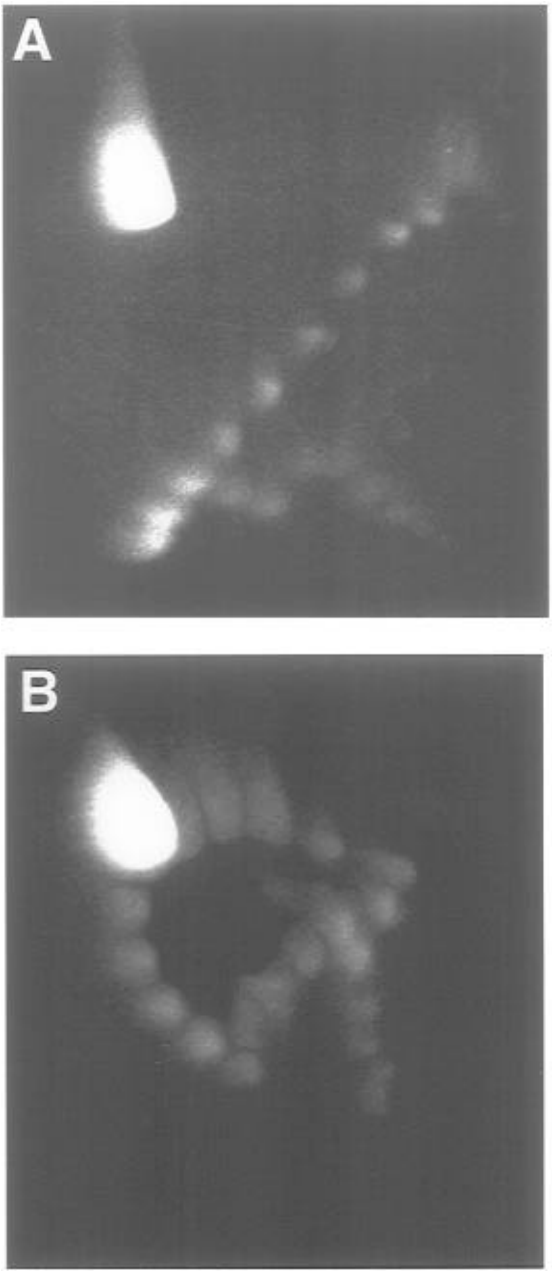

FIGURE 2: Two-dimensional gel-electrophoresis of a complete topoisomer population prepared from BS64: (A) standard conditions; (B) first dimension run in the presence of 150 μM Co(III). First dimension, top to bottom; second dimension, left to right. Preparation of topoisomers and gel-electrophoresis conditions are described in Experimental Procedures.

TG block (Ellison et al., 1987; Albert et al., 1994). The limited induction of Z-DNA that is observed even in topoisomers of high superhelical densities is, consequently, ascribed to the large energetic penalty associated with the formation of multiple BZ junctions.

A drastically different situation is encountered when the first dimension of the gel containing the topoisomers derived from BS64 is run in the presence of Co(III) (Figure 2B). On the basis of the position of the various topoisomers relative to the nicked-circular form it is evident that *all* topoisomers sustained significant relaxation. Apparently, three modes of conformational changes are induced. A non-cooperative B-to-Z transition occurring in all topoisomers is indicated by the reduced migration of all topoisomers and the appearance of positively supercoiled forms in the first dimension. A second, cooperative, transition occurs at topoisomer $\Delta Lk = -8$ (superhelical density of -0.028), resulting in an almost complete relaxation. In addition, a B-to-Z transition that is initiated at $\Delta Lk = -8$ is observed, leading to a progressive relaxation as the difference in the linking number is increased. All of these conformational changes are suggested to be supercoiling-mediated but significantly promoted by the metal ions.

Circular Dichroism Studies. The optical properties exhibited by the closed-circular plasmids BS, BS32, BS64, and BS76, as well as by their linearized derivatives, have been investigated at various conditiona previously shown to affect packaging of linear DNA into chiral aggregates (Eickbush & Moudrianakis, 1978; Maestre & Reich, 1980; Huey & Mohr, 1981; Shin & Eichhorn, 1984). Under all packaging conditions examined, the closed circular parent plasmid as well as the constructs containing the alternating purine−pyrimidine segments exhibit *negative* non-conservative ellipticities (Figure 3). In sharp contrast, the sign of CD signals exhibited by the chiral condensed phases of the linearized forms that are obtained from the various plasmids is found to depend upon the specific packaging system, i.e., the type and concentration of the salt. A clear trend in the relative magnitudes of the ellipticities exhibited by the assemblies that are formed from the closed supercoiled DNA molecules is observed: the signals revealed by the aggregates derived from the parent BS are consistently smaller than those characterizing the condensed species of the constructs containing alternating purine−pyrimidine inserts. Moreover, the magnitude of the non-conservative CD signals correlates with the length of the insert: larger signals are consistently exhibited by the chiral organizations that are obtained from plasmids with longer alternating purine−pyrimidine segments. The correlation between the magnitude of the non-conservative ellipticities and the presence or length of the inserts is underlined by its absence in the chiral aggregates derived from the linearized forms of the parent and recombinant plasmids: under a given set of packaging conditions all linear DNA molecules present identical non-conservative CD signals (Figure 3).

Thus, supercoiled DNA molecules form chiral aggregates whose optical parameters are dictated by *intrinsic* factors, i.e., the presence and size of the alternating purine−pyrimidine segments. Modifications of the packaging conditions result only in marginal alterations of the optical, and hence structural, properties. In contrast, the sign and magnitude of the ellipticities exhibited by condensed phases obtained from linear derivatives of the plasmids are completely independent of the presence or length of the inserts, being determined solely by the particular packaging conditions.

The prominent effects of the alternating purine−pyrimidine inserts upon the condensed phases of closed-circular plasmids are further manifested when the various chiral assemblies are studied in the presence of a DNA intercalating drug. Four distinct modifications of the non-conservative CD signals exhibited by the various aggregates can be discerned as the drug-to-DNA molar ratio is progressively increased (Figure 4). The aggregates obtained from the parent BS display a substantial increase of the magnitude of the negative ellipticity that is followed by a decrease to very low levels, indicating a complete disruption of the long-range order. Progressive addition of the drug to the supramolecular

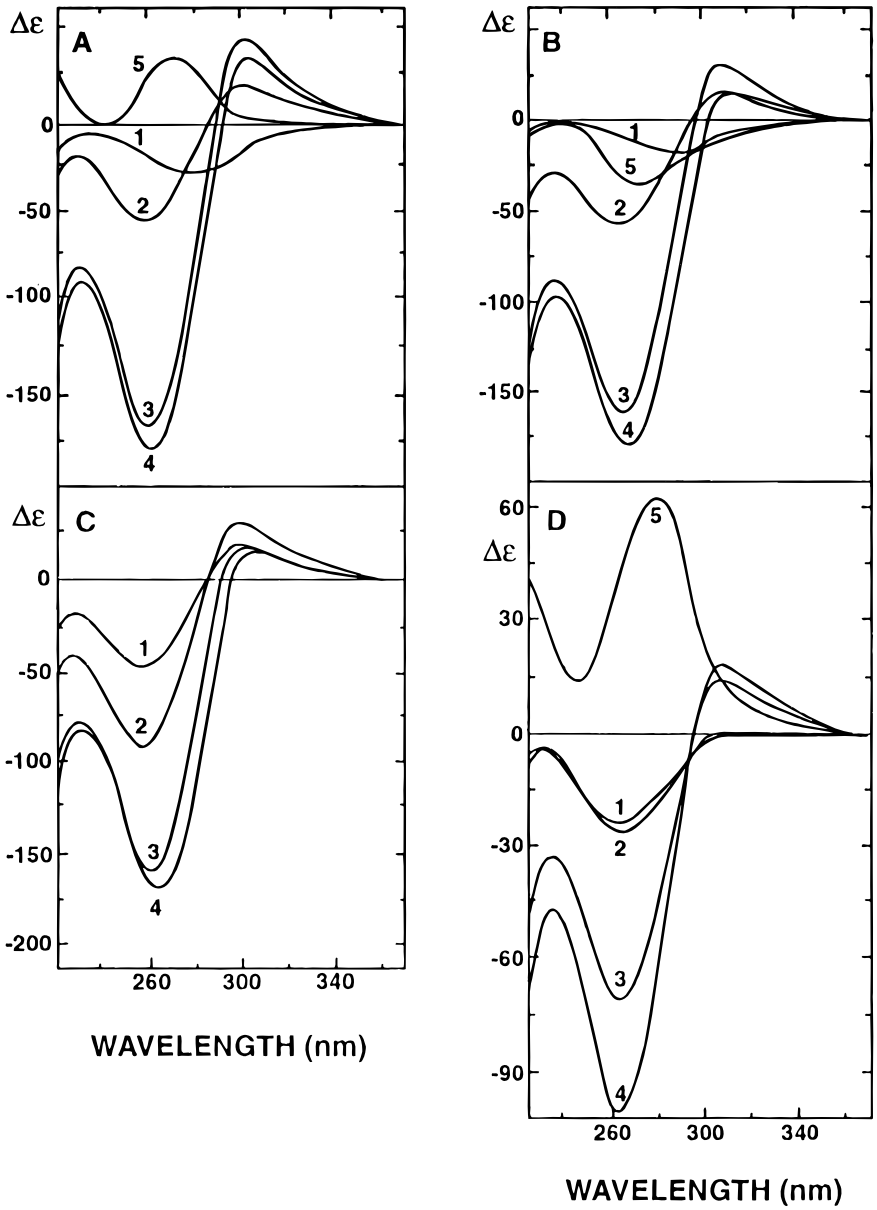

FIGURE 3: Circular dichroism spectra exhibited by the chiral assemblies derived from (1) BS, (2) BS32, (3) BS64, (4) BS76, and (5) linear derivatives obtained from each one of the plasmids. Packaging conditions were (A) 0.8 M NaCl, 35% EtOH; (B) 2.4 M NaCl, 35% EtOH; (C) 10 mM $MgCl_2$, 20% EtOH; and (D) 0.8 M $NaClO_4$, 35% EtOH. DNA concentration (in base pair molarity), 5×10^{-5} M. Samples were monitored at room temperature and pH 7.0.

conformations derived from both BS32 and BS64 results in the appearance two "waves" of increasing magnitude of the negative non-conservative CD, that are separated by a well where the size of the ellipticities decreases. A further increase of the drug-to-DNA molar ratio results in the collapse of the long-range order. Titration of the packed species obtained from BS76, which exhibit a very large CD signal in the absence of the drug, results in an initial decrease in the CD amplitude which is followed by an increase and finally by a drop to a conservative level. In clear contrast, the supramolecular conformations that are derived from linear DNA molecules obtained from the parent BS as well as from the various recombinant plasmids reveal an identical response to a titration with the intercalating drug: a slight initial increase of the CD signals followed by a decrease to very low values.

X-ray Scattering Measurements. The structural effects associated with the presence of alternating purine-pyrimidine inserts are clearly indicated by X-ray diffraction patterns obtained from concentrated phases of BS and BS64. X-ray scattering experiments were performed on samples prepared at two DNA concentrations, i.e., 25 and 75 mg/mL in 10 mM $MgCl_2$. Diffraction maxima (Table 2) point toward two conspicuous trends. As the DNA concentration is increased, the diffraction peak shifts toward higher scattering angles, implying shorter interplasmid separations. A line-width analysis of the peaks (Henry et al., 1961), indicates a minimum effective coherence length of 520 Å for the condensed DNA phases at both low and high DNA concentrations, thus suggesting that the observed signals correspond to interplasmid, as opposed to interhelical, spacings. This assumption is further supported by comparing the diffraction peaks to previously reported values obtained from condensed plasmid phases (Torbet & Dicapua, 1989).

The second trend clearly manifested by the results presented in Table 2 is related to the difference in the interplasmid spacings that are revealed by the condensed phases of the parent BS and the recombinant BS64: at both

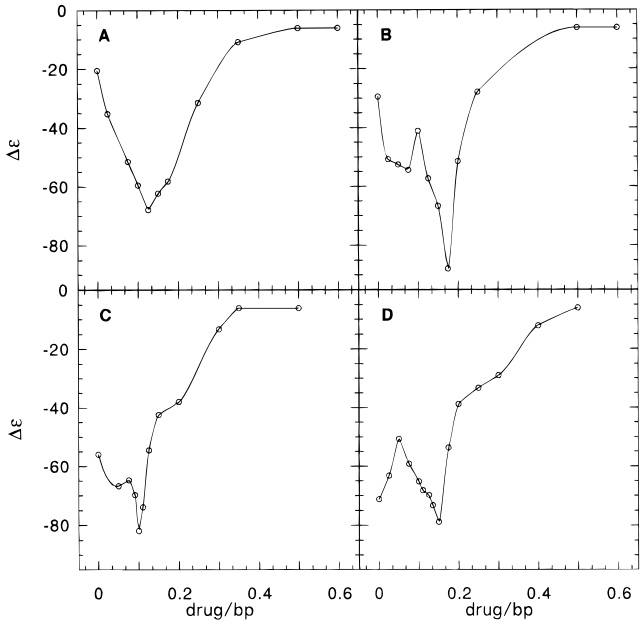

FIGURE 4: Ellipticity maxima exhibited by the plasmid assemblies in the presence of ethidium bromide at 10 mM $MgCl_2$, 20% EtOH, as functon of drug-to-base pair ratio; (A) BS; (B) BS32; (C) BS64; and (D) BS76. *The DNA concentration in all samples was 5 × 10^{-5} M, in base pairs.* Notably, the indicated drug-to-base pair ratio values represent the ratio of *included* drug to DNA and not of bound drug; the binding ratios could not be measured because, under packaging conditions, the system is not in equilibrium.

Table 2: Diffraction Maxima (in Å) Displayed by BS and BS64

plasmids	25 mg of DNA/mL	75 mg of DNA/mL
BS	105	71
BS64	130	80

DNA concentrations studied, a substantially larger spacing is observed for the BS64 plasmid which contains the alternating purine−pyrimidine segment. Notably, since both the ionic strength and DNA concentration are the same, the observed difference in the interplasmid spacings can be assigned only to a difference in the diameter of the two plasmids.

DISCUSSION

A B-to-Z DNA transition induced solely by native superhelical density is likely to be limited to a few alternating purine−pyrimidine residues (Lilley, 1986). The transition is, however, significantly promoted by the combined effect of the supercoiling energy and the synergistic activity of high ionic strength and dehydration (Zacharias et al., 1982). Indeed, a progressive relaxation which occurs as the length of the alternating purine−pyrimidine segments increases is indicated by shadowing electron microscopy where sample preparation entails exposure of DNA to salts and EtOH. The relaxation is assigned to a progressively larger number of base pairs that undergo a B-to-Z transition in going from BS to BS76. Notably, a B-to-Z transition of both inserts in BS76 is implied by the enhanced relaxation of this plasmid relative to the BS64 (Figure 1). Electrophoretic mobilities further indicate an enhanced relaxation of the recombinant plasmids following exposure to high ionic strength (Figure 2).

DNA Secondary Conformational Motifs, Supercoiling, and Packaging. It has recently been reported that supercoiled DNA spontaneously forms a cholesteric phase in which the rod-like plasmids form layers that lie on top of each other in a twisted manner, thus forming a highly chiral assembly [Reich et al. (1994a,b) and considerations outlined under Theoretical Background]. In contrast to chiral mesophases derived from linear DNA molecules whose parameters are dictated predominantly by the dielectric properties of the medium, the properties of the plasmid-derived assembly are directly determined by the molecular superhelicity (Reich et al., 1994a,b). Specifically, it has been shown that, due to their right-handed superhelical sense, mesophases derived from native plasmid molecules exclusively adopt a left-handed cholesteric twist which is manifested by negative non-conservative CD signals. Moreover, extrinsic factors that modulate the superhelical density and handedness, such as the temperature, pH, or intercalating drugs, were found to correspondingly alter the pitch and twist of the plasmid-derived cholesteric organization (Reich et al., 1994a,b).

The results presented in this study indicate that *intrinsic* factors, i.e., DNA secondary conformational changes, crucially affect the properties of the DNA chiral mesophase as well. Particularly indicative is the observation that, under all conditions which induce a cholesteric organization of the plasmids, the magnitude of the negative non-conservative CD spectra progressively and significantly increases as the length of the alternating purine−pyrimidine inserts is increased (Figure 3). We interpret this observation as follows: as implied by electrophoresis and electron microscopy, high-salt and dehydrating conditions that induce DNA chiral packaging, substantially promote a B-to-Z transition within the alternating purine−pyrimidine inserts. Since the Z motif is induced in a progressively larger number of base pairs going from BS to BS76, a corresponding progressive relaxation of the plasmids occurs, resulting in an increase of the plasmid diameter (Boles et al., 1990). Notably, this process occurs not only in dilute solution (as clearly observed in the shadowing electron micrographs) but also when the plasmids form condensed assemblies, as indicated by X-ray scattering of packed phases of BS64 which exhibit a significantly larger interplasmid spacing, and hence a larger plasmid diameter than BS (Table 2). The increased plasmid diameter leads to a larger cholesteric pitch and hence to an increase of the absolute size of the CD spectra as the length of the alternating purine−pyrimidine inserts increases [Kim et al. (1986) and considerations outlined under Theoretical Background]. Thus, a direct supercoiling-mediated effect of the Z motif on the chiral DNA assembly is indicated. The notion that the altered features of the cholesteric mesophases are directly associated with modulations of the superhelical parameters is further supported by the observation that the CD spectra, and hence the supramolecular properties, exhibited by chiral assemblies of *linearized* plasmids are completely independent of the presence and length of the alternating purine−pyrimidine segments (Figure 3).

A clear demonstration of the correlation between the superhelical parameters and the properties of the cholesteric phase is provided by the titration of the plasmid supramolecular organization with ethidium bromide (Figure 4). The presence of the intercalating agent in increasing drug-to-DNA ratios results in three structural modifications: a progressive relaxation of the superhelicity, a destabilization of the Z motif

(Walker et al., 1985) and a disruption of the long-range cholesteric organization (Reich et al., 1990). The initial increase in the magnitude of the negative non-conservative ellipticities exhibited by the cholesteric organization of BS, BS32, and BS64 is ascribed to a progressively increasing cholesteric pitch which results from a larger plasmid diameter associated with a drug-mediated relaxation. The drug-induced destabilization of the Z motif, which results in an increase of the superhelical density and hence in a smaller plasmid diameter and smaller cholesteric pitch, is indicated by a decrease of the CD magnitudes revealed by the long-range organizations derived from BS32 and BS64. This decrease is followed by an increase caused by the progressive relaxation of the plasmids. A final decrease in the magnitude of the ellipticities to conservative values exhibited by cholesteric organizations of all plasmids indicates the elimination of the long-range order (Reich et al., 1990). The cholesteric phase derived from the highly relaxed BS76 fails to exhibit the initial increase of the absolute CD magnitude; presumably, in BS76 a minute amount of the intercalating drug is sufficient to destabilize the Z motif and hence to increase the superhelical density prior to the drug-induced relaxation that is observed as the initial step in other plasmid-derived cholesteric phases. The sequence-dependent and supercoiling-mediated effects of the drug upon the cholesteric phases derived from the closed circular DNA molecules are underlined by the identical, *sequence-independent* effects of the drug on the supramolecular assemblies that are obtained from the linear forms of the various constructs.

The interrelationship among DNA primary sequence, secondary transitions, and the tertiary superhelical conformation is well established. The current study indicates that this relationship extends over an additional structural level, namely, the quaternary, supramolecular DNA organization. Specifically, it is shown that the pitch of the cholesteric DNA phase derived from supercoiled DNA molecules is determined by the supercoiling parameters, which, in turn, are modulated by secondary conformational changes whose nature and extent are sequence dependent. We argue that the supramolecular assembly is not only determined by the superhelical features but may affect these features as well; the long-range order observed for the condensed DNA phase implies that within this phase dynamic processes such as branching are suppressed. In addition, the tightly packed phase is likely to shift the B-to-Z equilibrium towards the B motif, since the formation of Z-DNA is associated with relaxation and hence a larger plasmid diameter. The observation according to which a packed cholesteric DNA organization is effectively regulated by superhelical parameters, and hence by cellular factors, indicates that such an organization might offer a particularly efficient packaging mode for nucleosome-free DNA molecules *in vivo*.

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BI9525482