# Effects of Charge Modification on the Helical Period of Duplex DNA†

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ABSTRACT: Supercoiled enriched PM-2 DNA has been relaxed by treating with calf thymus topoisomerase I and used in the preparation of a family of *n*-butylamine adducts of varying levels of substitution. The amine is cross-linked by formaldehyde to the exocyclic amino group of G when the DNA is in duplex form. These amine adducts of covalently closed relaxed (ccr) DNA, freed of the formaldehyde and *n*-butylamine reactants, have circular dichroism (CD) spectral properties similar to those previously reported for the adducts of calf thymus DNA [Chen, C., Kilkuskie, R., & Hanlon, S. (1981) *Biochemistry 20*, 4987–4995]. In both instances, the CD transformation effected by increasing levels of substituted cationic amine is similar to that induced by solvents of high electrolyte content. The adducts also exhibit greatly increased electrophoretic mobility compared to unreacted controls or a control treated only with formaldehyde. Mobility changes in the presence of variable amounts of ethidium bromide demonstrate that this phenomenon is attributable to the formation of negative supercoils and is not due to denaturation or unwinding of the duplex. Incremental increases in superhelicity due to the attachment of the amine have been measured by reference to a topoisomerase ladder of underivatized PM-2 DNA and converted to changes in winding angle. As the extent of substitution increases, the rotational strength of the positive band above 260 nm decreases, and the winding angle increases in the nonlinear manner observed previously for underivatized PM-2 DNA [Baase, W. A., & Johnson, W. C., Jr. (1979) *Nucleic Acids Res. 6*, 797–814]. In fact, the relationship between these two properties is the same for both the adducts and the underivatized ccr species. Thus, the attachment of the amine has the same conformational effects as the electrolyte content of the solvent. The effect can be rationalized in terms of the reduction of the electrostatic free energy of the duplex due to site-bound or localized cation binding in the minor groove.

The ability of nonintercalating electrolytes to influence the periodicity of the solvated DNA duplex has been studied extensively. Where direct measurements of winding angle have been made, the changes observed for random-sequence covalently closed DNA in aqueous solutions are small, amounting to no more than an average of 0.2–0.3 base pair (bp)[1] per turn at concentrations of monovalent salts near saturation (Wang, 1969; Anderson & Bauer, 1978; Baase & Johnson, 1979). It has been estimated that the maximum change induced in the DNA B structure by the ionic association of core histones can amount to no more than 0.2 bp per turn (Baase & Johnson, 1979).

The biological relevance of these relatively minor structural changes was not appreciated, however, until the advent of recent observations on the role played by the helical phase in regulating protein interactions and tertiary folding properties of the DNA duplex. Hochschild and Ptashne (1986), for instance, have demonstrated that functional interactions between regulatory proteins bound to DNA control elements can only be maintained when the protein binding sites on the duplex are located on the same face of the helix. The bending properties of small restriction fragments of the kinetoplasts of trypanosomes have been ascribed to the occurrence of blocks of four to six A's *repeated in phase with the helix* (Marini et al., 1982; Wu & Crothers, 1984; Kitchin et al., 1986). In addition, the overall tertiary structure of a covalently closed DNA species and the energy required to maintain the supercoiled state are intimately related to the helical periodicity. In the case of highly supercoiled structures, changes in twist in one segment may very easily lead to decreases in twist and secondary structure at other locations, thus changing structural surfaces presented to interacting proteins (Benham, 1979; Vologodski et al., 1979; Zacharias et al., 1982).

The origin of changes in helical periodicity is thus of interest when attempting to delineate regulatory mechanisms of gene expression. Really dramatic changes occur, of course, in the canonical secondary structural transformations, such as the B → Z and the B → A transitions, and the influence of electrolyte and solvent on these events has been extensively discussed (Behe et al., 1985; Pack & Klein, 1984; Pack et al., 1986). Of equal interest, however, are the more subtle changes which occur within the B family.

How the content and nature of the electrolyte induce changes of this latter type is unclear. The effectiveness of the various monovalent cations in increasing the winding angle of the DNA duplex parallels their ability to displace Na from the ion condensation layer of DNA (Bleam et al., 1980). It is not known, however, whether the displacing monovalent cation is actually site-bound to any extent. Theoretical treatments suggest that there exists nonuniformity in the localization of bound ions around the duplex, with the minor groove having a higher concentration of cations than the major groove in duplex B DNA (Pack & Klein, 1984). More recent elaborations of polyelectrolyte theory have suggested some degree of site binding, or more specific localization of counterions (Zimm & Le Bret, 1983; Matthews & Richards, 1984) and X-ray data on Cs DNA have revealed the presence of Cs ions bound directly to minor groove sites (Bartenev et al., 1983). It has not been demonstrated, however, that these

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[1] Abbreviations: bp, base pair(s); CD, circular dichroism; EDTA, ethylenediaminetetraacetic acid; crr DNA, covalently closed relaxed DNA; BuA, *n*-butylamine; EtdBr, ethidium bromide; Tris, tris(hydroxymethyl)aminomethane.

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associations persist in the solvated aqueous state.

In an effort to elucidate the nature of the interaction leading to an increase in winding angle of the B form duplex in electrolyte solutions, we have turned to an examination of the winding angle properties of a derivative of DNA in which positive charges have been specifically tied to the exocyclic amino group of the guanine nucleotides. We have previously demonstrated (Chen et al., 1981) that gem amine adducts of DNA can be created by cross-linking an amine to the exocyclic amino group of guanine via formaldehyde, with 1 mol of formaldehyde per mole of amine incorporated. A stable product, freed of unreacted formaldehyde and amine, can be isolated by exhaustive dialysis. The extent and rate of incorporation are dictated by the GC content of the DNA species with poly(dG-dC) exhibiting maximal uptake and poly(dA-dT) none at all (Maibenco et al., 1984). Identification of G as the stably modified site is evidenced by the fact that poly(dI-dC) fails to form a stable product and DNA previously modified with G specific reagents will take up less amine than unmodified controls under similar reaction conditions (Maibenco and Hanlon, unpublished results).

The product has a profoundly altered CD spectrum, with the magnitude of change paralleling the extent of derivatization. This alteration is related to the charge on the amine adduct rather than its steric properties since rapid titration to alkaline pHs will reverse the CD transformation without an appreciable loss of the adduct. The spectral alterations are not attributable to denaturation or transformation to a markedly different secondary or tertiary structure (such as a collapsed $\Psi$ structure) since the extinction coefficients, cooperative melting profiles, hydrodynamic properties, Raman spectra, and X-ray properties reveal that the DNA adducts have a base-stacked duplex structure with B form backbone geometry and are in a nonaggregated form in solution (Chen et al., 1981, 1983; Fish et al., 1983).

The CD spectra of a family of such derivatives containing different amounts of amine resemble those produced by the variable addition of high concentrations of simple monovalent alkali metal cations to neutral aqueous solutions of DNA. Baase and Johnson (1979) as well as our own laboratory (Chan et al., 1979) have demonstrated that the CD changes of unmodified DNA in electrolyte solutions are correlated with the average changes in the helical periodicity—or winding angle—of the DNA duplex. In fact, the changes in the rotational strength of the positive band above 260 nm in the near-UV CD spectrum are usually used as a diagnostic measure of changes in winding angle within B family structures (Belintsev et al., 1979). The quantitative relationship between the rotational strength of this band and the winding angle changes has also been shown to be independent of solvent: The dependence observed in mixed aqueous–ethanol solvents containing monovalent cations at ethanol concentrations below the B $\rightarrow$ A transition (Ringquist, 1987; Ringquist & Hanlon, 1986) is identical with that observed in aqueous electrolytes in the same winding angle range.

The similarity in the CD effects produced by increasing extent of amine substitution in the derivatives suggests that one of the secondary structural changes responsible for the CD spectral transformation in these derivatives is a change in winding angle. Since the CD changes in the derivative are related to the placement of the cationic charge at a specific location, the demonstration of winding angle changes in the derivatives might permit us to make some judgment about the general mechanism of cation-induced winding changes. In order to evaluate these possibilities, we have undertaken the following study on covalently closed PM-2 DNA adducts prepared with *n*-butylamine.

## Experimental Procedures

*Preparation of Calf Thymus Topoisomerase.* Because the attached amine in the derivatives is positively charged, we anticipated that the data from ethidium bromide titrations of winding angle changes would be difficult to interpret without extensive characterization of the binding characteristics and unwinding angles for the intercalation of ethidium into the derivatives. Because of this, the winding angle changes have been evaluated, predominantly, by the electrophoretic method of Shure and Vinograd (1976) using covalently closed PM-2 DNA, relaxed before reaction. The calf thymus topoisomerase I used in the preparation of the covalently closed relaxed DNA was isolated by the procedures of Pulleyblank and Morgan (1975) and Liu and Miller (1981). The initial extract was further purified by passage over a phosphocellulose column using an elution solvent of increasing NaCl content. Activity was followed by an assay in which one unit of topoisomerase activity was defined as the amount of enzyme required to relax 1 $\mu$g of form I DNA in 30 min at 25 °C in the assay buffer. The assay buffer was 200 mM NaCl, 10 mM $NaH_2PO_4$/$Na_2HPO_4$, and 2 mM EDTA, pH 7. The extent of relaxation was determined by electrophoresis on 0.7% agarose gels as described below.

*Preparation of Covalently Closed Relaxed PM-2 DNA.* PM-2 phage, grown on its host, *Alteromonas espejiana*, was isolated by the method of Salditt et al. (1972). (The original stocks of the phage and its host were gifts of Dr. Stuart Linn of the Department of Biochemistry, University of California at Berkeley.) PM-2 DNA was prepared from the phage according to the procedure of Espejo and Canelo (1968) as modified by Kovacic and van Holde (1977). The DNA obtained at this stage was ca. 75% form I (native supercoiled) and 25% nicked form II (relaxed) with negligible amounts of form III (linear). The preparation was enriched for form I by the acid phenol extraction procedure of Zasloff et al. (1978). Concentrations of all DNA solutions were determined by absorbance spectroscopy, using an extinction coefficient of 6600 $M^{-1}$ $cm^{-1}$ (Baase & Johnson, 1979).

This supercoiled enriched preparation was relaxed at 25 °C by calf thymus topoisomerase I, using 1–2 units of enzyme per microgram of DNA in assay buffer for 30 min to 4 h. The protein was then removed by treatment with proteinase K followed by phenol extraction and dialysis against 200 or 20 mM NaCl–10 mM $NaH_2PO_4$/$Na_2HPO_4$ buffer at pH 7.0. Although the supercoil enrichment step was effective in decreasing the fraction of nickel form II to ca. 5%, further manipulations to produce this covalently closed relaxed (ccr) product resulted in a final nicked relaxed population of 10–15% as estimated from the gel electrophoresis experiments. Relaxation was never complete, and the "relaxed" preparations contained anywhere from 5% to 10% of the DNA in the native supercoiled form.

*Preparation of the n-Butylamine Derivatives of Covalently Closed PM-2 DNA.* Adducts of PM-2 DNA, formaldehyde, and *n*-butylamine were prepared by the procedure of Chen et al. (1981). A solution of DNA at ca. 0.1 mM in 200 or 20 mM NaCl–10 mM $NaH_2PO_4$/$Na_2HPO_4$ was mixed with formaldehyde and *n*-butylamine to give a final concentration of reactants of 2% formaldehyde and 2.5, 5, or 10 mM *n*-butylamine. A control solution containing only DNA and formaldehyde was simultaneously prepared, and the two solutions, the experimental reaction mixture and its control, were monitored by CD and absorbance spectroscopy as a function

of time up to 2–2.5 h. Within this time frame, the absorbance of the DNA maxima did not exceed 2% of their initial unreacted values, indicating that the products in the reaction mixtures were not undergoing denaturation or significant base unstacking during the course of the reaction. In order to obtain derivatives with variable amine content, aliquots were removed at different times during the reaction. In most instances, they were dialyzed exhaustively against 20 mM NaCl–10 mM $NaH_2PO_4/Na_2HPO_4$, pH 7, to remove unreacted reagents. In a few cases where the reaction products were examined directly on gels without dialysis, the aliquots were frozen in a dry ice–acetone bath and held there until the final reaction aliquot had been collected. This freeze/thawing procedure generally resulted in excessive nicking. This would not have affected the CD spectral preparations which were monitored continuously throughout the reaction in a separate aliquot.

We have previously shown for both random-sequence linear DNAs and poly(dG-dC) that variable amounts of attached amine correlate in an approximately linear fashion with the characteristics of the CD spectrum of the dialyzed product (Chen et al., 1983, 1987). The relationship between the fractional amount of derivatization, $R$, in moles of amine per mole of nucleotide, and the mean residue ellipticity at 275 nm, $[\theta]_{275}$, is dependent on the GC content but is insensitive to the source of DNA (procaryotic or eucaryotic) (Maibenco and Hanlon, unpublished results). Over the range of $R$ values between 0 and 0.15, the value of $R$ can be calculated from the equation:

$$R = m([\theta]^0_{275} - [\theta]^R_{275}) \quad (1)$$

where $[\theta]^0_{275}$ is the mean residue ellipticity at 275 nm of the underivatized control and $[\theta]^R_{275}$ is the mean residue ellipticity for the derivatized product with $R$ moles of amine bound per mole of nucleotide. We have used this to evaluate the amine content of PM-2 DNA. The value of the slope term, $m$, was taken as $1.34E - 5$, the value previously determined for calf thymus DNA whose GC content is that of PM-2 DNA (42% GC).

*Determination of Changes in Superhelicity in PM-2 DNA.* The supercoil content of PM-2 DNA derivatives prepared from covalently closed relaxed DNA was estimated by using the band-counting method of Shure and Vinograd (1976). Electrophoresis of PM-2 DNA was performed at 25–27 °C on 0.7% agarose slab gels in TEA buffer (40 mM Tris–acetate, 20 mM sodium acetate, and 2 mM NaEDTA, pH 8.3) using an electrophoresis apparatus, Model H1 from Bethesda Research Laboratories. A voltage gradient of 2 V/cm was applied for 16–20 h. Loss of amine from dialyzed controls allowed to stand on the bench top under the same conditions was negligible, as ascertained by examination of the CD properties of the solution. The DNA bands were visualized by ethidium bromide staining and illumination on a UV light box at 320 nm. The gels were photographed, and the negatives were scanned with either an E.C. Corp. or a Beckman Instruments Analytical densitometer. Because of the random dispersion of substituted sites among the various topoisomers of the relaxed population, the derivatives did not exhibit clearly resolved topoisomer bands. The centers of the broad bands seen in these experiments were used as the position of the population average. The number of supercoils was calculated by dividing the distance between this position and the center of the band corresponding to the nicked relaxed form II by the average supercoil spacing appropriate for the given experiment. The latter was obtained by running a partially relaxed sample of PM-2 DNA, containing multiple topoisomers, in one of the lanes. The use of the position of the nicked form II corrected for the slight decrease in mobility caused by the reduction in the negative charge of the DNA by the substituted positively charged amine. The number of supercoils obtained from this measurement was corrected for the 3.0 positive supercoils introduced by the transfer from relaxation buffer (200 mM in Na) to the lower cation content electrophoresis buffer (ca. 50 mM Na). For the low superhelical densities of the DNA species examined in these experiments, the number of superhelical turns ($\tau$) is equal to the writhe function (Wr), and the relationship to twist (Tw) in these experiments is simply

$$\Delta\tau = -\Delta\mathrm{Tw} \quad (2)$$

The average change in winding angle for the 9850 bp PM-2 DNA was calculated from the $\Delta\tau$ values using the relationship:

$$\Delta\Psi = -\Delta\tau(360/9850) \quad (3)$$

Winding angle changes for the underivatized ccr DNA in electrolyte solvents were taken from the literature. Values in dilute salt solutions were the data of Anderson and Bauer (1978). Values for the more concentrated solvents, 3.0 M CsCl, 6.2 M LiCl, and 5.4 M $NH_4Cl$, were those given in Table II of Baase and Johnson (1979). We noted that the experimental values in the CsCl and the $NH_4Cl$ solvents corresponded to the predicted value using the logarithmic relationships of Anderson and Bauer (1978) for these salts. We therefore used these relationships to calculate the winding angle changes for 4.7 M CsCl and 3.5 M $NH_4Cl$ for which direct experimental measurements were not available. All winding angle data were corrected for temperature differences using the coefficient of DePew and Wang (1975).

*Ethidium Bromide Titrations of DNA.* Native form I, derivatized ccr PM-2 DNA with 0.1 mol of amine per mole of nucleotide, and its formaldehyde control were titrated with ethidium bromide, using the tube gel procedure of Espejo and Lebowitz (1976) in which each tube contains a different concentration of ethidium bromide. Titrations of calf thymus DNA derivatized to the extent of 0.11 and 0.09 mol/mol of nucleotide, together with the appropriate controls, were also conducted spectroscopically, using the conditions of Crawford and Waring (1967). Extinction coefficients for free ethidium reported in that paper were used in our experiments. The extinction coefficients of the bound ethidium were determined by the saturation limits of the spectral shifts. The ethidium bromide (Sigma) used in these titration experiments was recrystallized from ethanol. Data from the formaldehyde controls and the unreacted DNA samples were indistinguishable. The two calf thymus DNA–BuA derivatives exhibited a different titration pattern. For the purposes of our experiments, the spectrophotometric titration data were, in general, not analyzed in terms of affinity constants and site exclusion parameters but were simply used to assess moles of ethidium bound by the DNA sample at a given concentration of free ethidium. In the concentration range of interest, however, the binding data plotted in the form of a Scatchard plot could be represented by a single affinity constant, $k$, and maximal moles of ethidium bound per mole of nucleotide, $\nu_n$. The affinity constant for the derivatives in this range was that for the controls (underivatized calf thymus DNA and the formaldehyde control), but the value of $\nu_n$ was significantly reduced from 0.18 to 0.10. For the calculation of winding angle changes in the derivatized ccr samples in the gel electrophoresis experiments, these calf thymus DNA together with an assumed unwinding value of 26° (per mole of ethidium per mole of nucleotide) (Wang, 1974) were used.

*Spectroscopy.* Absorption spectra were obtained with a

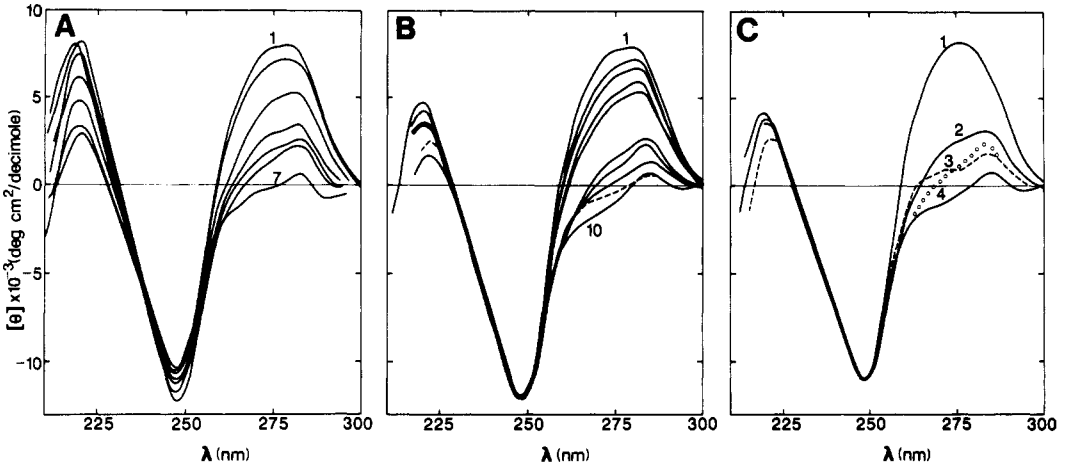


FIGURE 1: Cation effects on the CD spectral properties of PM-2 DNA. (A) CD spectra of covalently closed relaxed PM-2 DNA with different amounts of attached BuA. Curves 1–7 represent increasing levels of substitution of amine. Spectrum 1 represents 0 mol, and the other curves fall in order of decreasing rotational strength, above 260 nm, as 0.012, 0.040, 0.065, 0.077, 0.087, and 0.108 mol/mol of nucleotide, which is spectrum 7. The solvent in all cases is 20 mM NaCl–10 mM $NaH_2PO_4/Na_2HPO_4$, pH 7. (B) CD spectra of covalently closed relaxed PM-2 DNA in various salt solutions. Curves 1–10 represent solvents of different ionic content. Spectrum 1 is 0.02 M NaCl, and the other curves fall in order of decreasing rotational strength, above 260 nm, as 0.10 M KCl, 0.30 M KCl, 0.10 M $NH_4Cl$, 0.30 M $NH_4Cl$, 3.00 M CsCl, 3.50 M $NH_4Cl$, 4.9 M CsCl, 6.4 M LiCl (dashed curve), and 5.4 M $NH_4Cl$, which is spectrum 10. All solutions were buffered with 10 mM $NaH_2PO_4/Na_2HPO_4$, pH 7. The spectrum in 6.4 M LiCl (9) is dashed to facilitate comparison with the others in the panel. (C) CD spectra of native supercoiled PM-2 DNA in various salt solutions. Curves 1–4 represent solvents of 0.02 M NaCl (1), 2.8 M CsCl (2), 6.0 M LiCl (3), and 5.45 M $NH_4Cl$ (4) with 10 mM $NaH_2PO_4/Na_2HPO_4$, pH 7. The unnumbered beaded curve is a spectrum of covalently closed relaxed PM-2 DNA in 3.5 M $NH_4Cl$ from panel B, added for comparison.

Cary Model 14 recording spectrophotometer at 25–27 °C in 0.1–1.0-cm quartz cells also employed in the CD experiments. Values at 259 nm were used routinely to estimate DNA concentrations, using an extinction coefficient of 6600 $M^{-1}$ $cm^{-1}$ for PM-2 DNA (all forms) (Baase & Johnson, 1979) and calf thymus DNA. CD spectra were obtained on the same solutions on two instruments, a Cary 60 spectropolarimeter equipped with a 6001 CD attachment and a Jasco Model J40. Both instruments were calibrated with the same solution of *d*-10-camphorsulfonic acid. Spectra of the same DNA solution run on the two instruments were found to be identical within experimental error ($\pm 0.2E + 3$). CD spectra were analyzed by procedures previously reported (Hanlon et al., 1975; Wolf et al., 1977). The results are reported as mean residue ellipticities. Procedures for the assessment of the number of independent spectral components were those used in Wolf et al. (1977).

*Chemicals and Solutions.* All inorganic chloride salts were reagent-grade Fisher products. DNA solutions containing high concentrations of CsCl, $NH_4Cl$, and LiCl were prepared by the addition of concentrated stock electrolyte solutions to the appropriate DNA solution on ice. Concentrations of the electrolyte were checked by measuring the value of the refractive index at the Na D line, using a Bausch and Lombe Abbe refractometer. Solutions were kept tightly stoppered, and transfer to spectrophotometer cells was made as rapidly as possible in order to avoid dilution with atmospheric water.

## Results and Discussion

Upon mixing covalently closed relaxed (ccr) PM-2 DNA with *n*-butylamine and formaldehyde, the CD properties were observed to transform in the manner previously observed for calf thymus DNA (Chen et al., 1981). After exhaustive dialysis to remove excess reagents, the general character of the transformation was retained, with the most dramatic effect being the lowering of the rotational strength of the positive CD band above 260 nm in the reaction mixtures containing *n*-butylamine. If aliquots were removed from the reaction mixture at different times during the course of the reaction, the resulting dialyzed products exhibited a family of spectra of the type shown in Figure 1A, for ccr relaxed DNA. As had previously been observed for calf thymus DNA, the spectral properties of the reaction mixtures and the dialyzed products were very similar as long as the reduction in the magnitude of the positive band corresponded to values of $[\theta]_{275}$ of $5E + 3$ deg $cm^2$ $dmol^{-1}$ and above. Below that value, dialysis resulted in significant increases in the value of the rotational strength of this positive band in the final dialyzed adduct. The products of the reaction with native supercoiled DNA exhibited a similar pattern, although the same reaction conditions resulted in less extreme lowering of the positive band and more dramatic changes upon dialysis. Studies with other DNAs and synthetic polynucleotides reveal that this family reflects variable amount of attached amine. The level of substitution for each is given in the figure legend.

Figure 1B shows the similarity between the effects of the amine and those of increasing concentrations of neutral electrolyte. With the exception of the concentrated LiCl solution (6.4 M), the spectra displayed in Figure 1B also appear to form a similar family. The minimal difference observed in the relaxed species in concentrated LiCl becomes highly exaggerated in the spectra of the native supercoiled form I DNA in the same solvent. This point is demonstrated in Figure 1C, in which the spectrum of the supercoiled form in 6.0 M LiCl is shown as the dashed line, 3. In order to more clearly identify the deviations of the CD spectrum of the latter, we have also displayed the spectrum of the ccr DNA in 3.5 M $NH_4Cl$ (unnumbered beaded line), a solvent whose rotational strength of the positive band is similar. Comparison of the spectra displayed in the two panels of this figure, B and C, also demonstrates the observation made by others (Maestre & Wang, 1971; Belintsev et al., 1979) that the response of the rotational strength to the ionic content of the solvent is always less for the supercoiled form than that of the linear or relaxed forms.

The major differences in the two spectral families (salt and BuA derivatives) are exemplified by spectra 1 and 2 in Figure 2A. Although the rotational strengths of the positive bands are roughly comparable, the amine derivative has lower values of the mean residue ellipticities above 280 nm and higher

Table I: Supercoiled Characteristics of PM-2 DNA As Assayed by Ethidium Titrations Using the Espejo–Lebowitz Method

| sample | [EtdBr] at min mobility (μg/mL) | $\nu_c$[a] | $\tau$ | $\nu_c$[b] | $\tau$ |
|---|---|---|---|---|---|
| native form I | 0.20 | 0.073 | −104 ± 10 | | |
| form I–BuA adduct (ca. 0.1 mol of BuA/mol of nucleotide) | ~0.6 | ~0.11 | −150 ± 20 | ~0.07 | −81 ± 20 |
| ccr form II–BuA adduct (ca. 0.10 mol of BuA/mol of nucleotide) | 0.054 | 0.028 | −40 ± 5 | 0.015 | −21 ± 5 |

[a] Binding characteristics of underivatized PM-2 DNA taken from Espejo and Lebowitz (1976): $k = 13.3 \times 10^5$ M$^{-1}$; $\nu_n = 0.18$. [b] Average of data from two samples of calf thymus DNA: $k = 13 \times 10^5$ M$^{-1}$; $\nu_n = 0.10$.

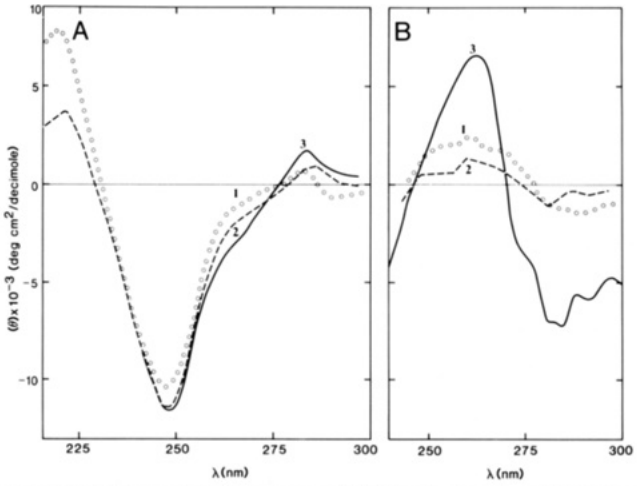


FIGURE 2: CD spectral differences at high levels of amine substitution and in concentrated electrolyte solvents. (A) Spectra 1 (O) and 2 (---) represent the spectrum of covalently closed relaxed PM-2 DNA with 0.11 mol of amine/mol of nucleotide and that of the underivatized sample in 5.45 M $NH_4Cl$, respectively. Spectrum 3 (—) is the linear extrapolation of the CD properties in the salt solutions in which the induced winding angle change was 0.4° or less. (B) Spectra 1 (O) and 2 (---) represent the differences between the spectra of the amine derivative (1) or the high salt solution (2) and the linear extrapolation shown in panel A. Spectrum 3 in this panel B is the spectral component extracted from the high salt effects on calf thymus DNA (Hanlon et al., 1975).

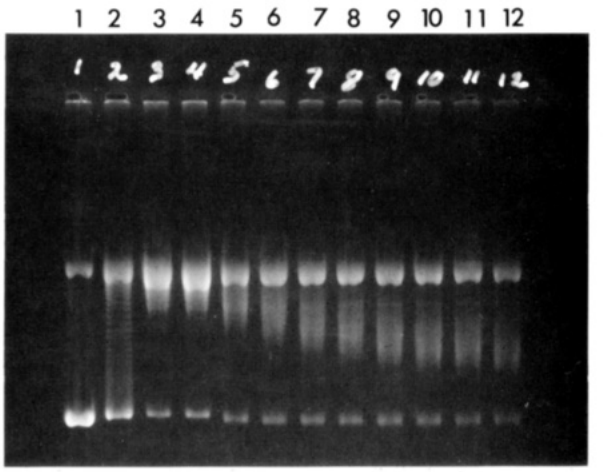


FIGURE 3: Effects of different levels of amine substitution on the supercoiled content of PM-2 DNA. The lanes are numbered sequentially from left to right. Lane 1 contains the supercoiled enriched PM-2 DNA preparation before relaxation with calf thymus topoisomerase. Lane 2 is a partially relaxed sample showing the topoisomer ladder used in the estimation of the number of supercoils. Lane 3 contains the completely relaxed preparation used in the chemical substitution reaction. Lane 4 represents the formaldehyde control, and lanes 5–12 contain the derivatives removed from a reaction mixture containing 10 mM BuA and 2% formaldehyde at 10, 25, 40, 55, 80, 95, 110, and 113 min, respectively. Samples were maintained at −60 °C until loading. CD spectral properties revealed that they contained 0.023, 0.034, 0.045, 0.058, 0.069, 0.080, 0.081, and 0.084 mol of amine/mol of nucleotide, respectively.

values below 280 nm. This is very similar to the differences seen in the spectrum of the unmodified DNA in the concentrated LiCl solvent, when the spectrum is compared with one of comparable rotational strength of the positive band. In the derivatives, however, there is an additional difference. The low-wavelength positive band at ca. 220 nm is almost double in intensity. Although this enhancement of the band at 220 nm frequently appears in denaturation, we wish to emphasize that the derivatives of PM-2 DNA are not denatured. Extinction coefficients and hyperchromic changes upon cooperative melting of BuA derivatives have always been found to be identical with the unreacted controls.

When either the reaction mixtures or the dialyzed products were electrophoresed, the mobility of the formaldehyde controls (subjected to the same reaction conditions in the absence of amine) were not different from those of the unreacted controls. In contrast, the mobilities of both supercoiled and ccr DNA which had been exposed to reaction mixtures containing *n*-butylamine were increased. Figure 3, which displays the patterns for a set of derivatives with different levels of substitution, shows that the increase was particularly dramatic for the ccr species, but a small increase can also be observed for the native supercoiled species whose content was ca. 10% in these preparations.

It could be shown that the increases in mobility of the covalently closed DNA species observed in Figure 3 reflected changes in the negative supercoiling of the PM-2 DNA species and were not a result of the collapse of the species to denatured forms, or the conversion to positive supercoiled species due to the local denaturation of segments of the duplex structure. This latter was an unlikely explanation since both ccr and native supercoiled DNA exhibited a mobility increase. In order to definitively lay this possibility to rest, however, we conducted a crude ethidium bromide titration using the electrophoretic method of Espejo and Lebowitz (1976). A typical set of results for one of the derivatives whose level of substitution is 0.10 mol of amine/mol of nucleotide is shown in Figure 4. As the concentration of ethidium bromide in the gel tubes increased, the mobility of the derivatized DNA at first decreased, went through a minimum, and then began to increase. Such behavior can only be explained by the intercalation of ethidium bromide into, initially, a negatively supercoiled species, causing unwinding of the duplex and ultimately resulting in the production of positive supercoils. Table I gives the ethidium bromide concentrations at the minimum mobility for the derivatized product of ccr PM-2 DNA, a derivatized product of native form I PM-2 DNA, and underivatized native form I PM-2 DNA.

The results for underivatized form I PM-2 DNA agree reasonably well with that observed by Espejo and Lebowitz (1976), who found that $\nu_c$ was 0.063, corresponding to ca. −90 superhelical turns in form I PM-2 DNA under these experimental conditions. The value of the critical relaxing concentration is excessively high, however, for the derivative of supercoiled PM-2 DNA and appears to be anomalous for the ccr derivative whose pattern is shown in Figure 3. If these derivatives exhibited the same binding characteristics as un-

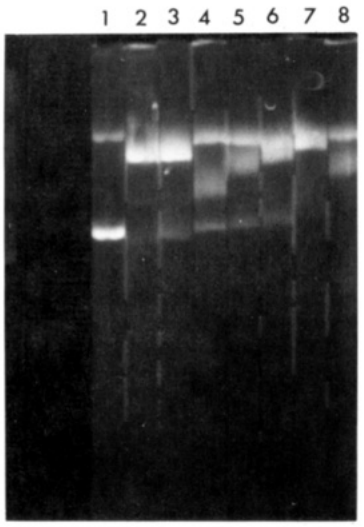


FIGURE 4: Demonstration of the negative supercoiled character of the amine derivatives of PM-2 DNA. The tube gels are numbered sequentially from left to right. Contents of tubes 1–8 are as follows: native form I (fast component) and nicked form II (1); covalently closed relaxed form II mixed with nicked form II (2); formaldehyde-treated control of sample run in tube 2 (3); amine derivative at 0.10 mol of amine/mol of nucleotide (4); tubes 5–8 contain the amine derivative shown in tube 4 with 0.011, 0.021, 0.054, and 0.107 $\mu$g of EtdBr/mL, respectively. Tube 7 with 0.054 $\mu$g/mL has been taken as the totally relaxed end point.

modified PM-2 DNA, the ccr derivative would have ca. −40 superhelical turns, and the derivative of native supercoiled PM-2 DNA would have −150, bringing the superhelical density of the latter to an astronomical level of −0.15.

The use of the binding characteristics of unmodified PM-2 DNA, however, is clearly unjustified. The amine modification which is positively charged would be expected to affect both the affinity constant and the near-neighbor site exclusion parameter. This prediction could be verified by spectrophotometric titration experiments with derivatives of calf thymus DNA at equivalent levels of substitution. The results of these experiments revealed that the binding of ethidium is markedly lower in the derivatives, compared to the controls at the same concentration of free ethidium, and the reduction in binding parallels the level of substitution. For the derivative whose data are displayed in Table I, the binding data taken from the calf thymus DNA studies on derivatives whose amine content brackets that of the derivative (0.11 and 0.09) indicate that the ccr derivative had 21 negative supercoils.

The attachment of the amine to PM-2 DNA thus increased the extent of negative supercoiling. Since the *relaxed* molecules had not been opened and then resealed, the changes in the level of supercoiling presumably reflect changes in the helical periodicity or twist. The mobility data of the type shown in Figure 3 were then used to calculate changes in winding angle, $\Delta\Psi$, for derivatives of ccr DNA. CD spectra on the same derivatives, some of which are displayed in Figure 1A, were also obtained. The correlation between these two properties is demonstrated in Figure 5A which also includes similar data obtained on the unmodified ccr samples in different electrolyte solvents. Winding angle data for the latter were taken from Anderson and Bauer (1978) and Baase and Johnson (1979), as described under Experimental Procedures. For comparison, ellipticity data from Baase and Johnson have been incorporated as well.

Despite the scatter of the experimental data, the correspondence between the two data sets, the ccr derivatives in the standard 20 mM NaCl–10 mM phosphate buffer and the unmodified ccr samples at variable concentrations of elctrolyte,

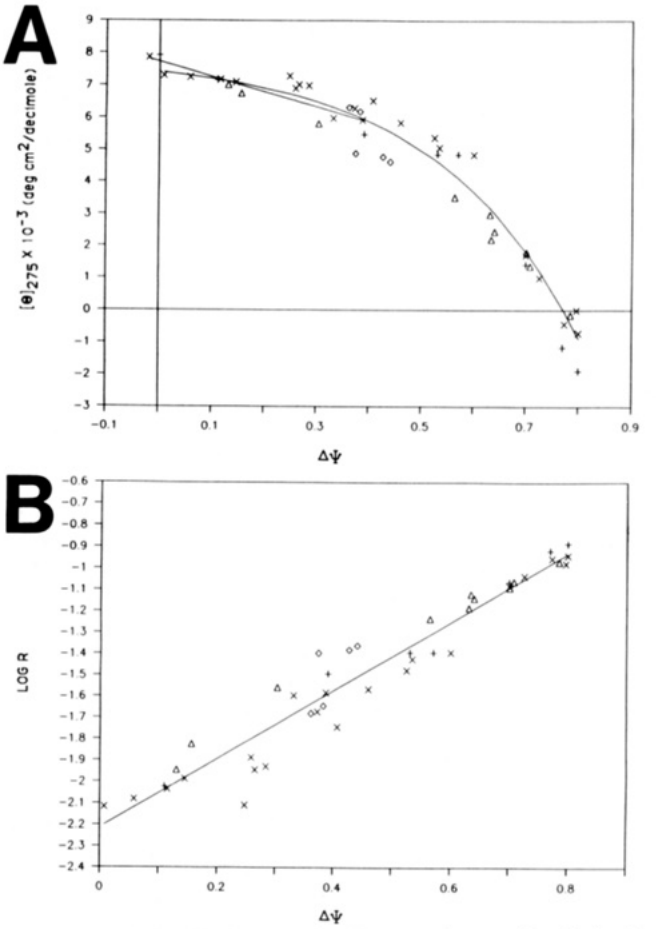


FIGURE 5: Relationship between the CD spectral properties, the level of amine substitution, and the changes in winding angle of covalently closed relaxed PM-2 DNA. (A) mean residue ellipticity at 275 nm for the dialyzed BuA derivatives (△); the amine reaction mixtures (◇), and the salt solutions (×). Data from Baase and Johnson (1979) are also included as the (+) points. The solid line drawn through the points represents the function arrived at by a linear regression of the data found in panel B. (B) Plot of the log of the cations bound to PM-2 DNA vs $\Delta\Psi$. Points are coded as in panel A. Values of $R$ are obtained for the various DNA species, as described in the text.

is striking. The wavelength chosen for this display was 275 nm in order to compare the data to those previously reported by Baase and Johnson (1979). As mentioned previously, the shape of the positive band above 260 nm in the salt and the derivative spectra vary somewhat, but the difference is small at this wavelength. The bias introduced has negligible effects on the pattern. Similar results were obtained using the rotational strength of this band (with integration limits between 260 and 300 nm).

The plot in Figure 5A appears to be nonlinear over the entire range of $\Delta\Psi$. The continuous line drawn through all the points is an exponential whose origin is explained in the next section. Data points at low winding angle changes (between $\Delta\Psi$ values of 0° and ca. 0.4°), however, can be approximated by the linear relationship

$$[\theta]_{275} = (-4.67E + 3)\Delta\Psi + 7.7E + 3 \qquad (4)$$

which describes the properties of the straight line drawn in Figure 5A. The slope of this relationship is essentially identical with that obtained by Chan et al. (1979) for calf thymus DNA in dilute electrolyte solvents ($-4.71E + 3$) and by Ringquist and Hanlon (1986) for covalently closed pBR322 DNA ($-4.3E + 3$) in mixed ethanol–aqueous solvents containing Na ions over the same range of winding angle changes. The curvature in this region of the curve, however, becomes apparent when

the limiting slope between −0.02° and 0.25° is calculated. This latter value is only about half as great, amounting to $-2.5E$ + 3 deg cm$^2$ dmol$^{-1}$ deg$^{-1}$.

In undertaking these experiments using ccr DNA, we had assumed that the superhelical density induced by the solvent and amine substitution would be low enough to allow the winding angle to assume the unperturbed value exhibited by the unconstrained linear form under the same environmental conditions. The conformity of the data of Baase and Johnson (1979) to the pattern exhibited by our data over most of the winding angle range confirms the validity of this assumption. At the very highest values of $\Delta\Psi$, however, it seems to fail. The differences observed in the values of $[\theta]_{275}$ in 6.2 M LiCl and 5.4 M $NH_4Cl$ at winding angles of ca. 0.8° are outside our joint experimental errors for the spectral determinations and are unlikely to be due to systematic errors since our values agree (within the limitations of our experimental errors) at all other lower electrolyte concentrations. The deviations in the values of $[\theta]_{275}$ are also larger than can be accounted for by the 10% or less contamination of form I. Assuming that values of $[\theta]_{275}$ reflect only winding angle, the nature of the deviation would suggest that the linear PM-2 DNA can undergo a slightly higher winding angle change (by ca. 0.05°) than can unmodified ccr in response to these concentrated electrolyte solvents (6.2 M LiCl and 5.4 M $NH_4Cl$). This is surprising in view of the fact that the maximal superhelical density of the ccr species in these solvents amounts to no more than −0.022. One would not have anticipated that such a low superhelical density would affect the ability of the duplex to take up its minimal energy secondary structure. This effect implies that the winding angle change of the last two points for the unmodified ccr samples in electrolyte solvents may have a systematic error of ca. 5%. The data for the derivatives, however, are unaffected since the values of $\Delta\Psi$ for those samples were measured directly and were not taken from the literature.

The nonlinear relationship between the rotational properties and the winding angle in the two data sets, the BuA and the salt data, is puzzling. The average winding angle change which is sufficient to reduce the rotational strength of the positive band to ca. 0 is 0.8°. It is strange that the function relating the CD properties to the winding angle of the duplex structure exhibits nonlinear behavior within so small an angular range. This observation is also inconsistent with the theoretical calculations and predictions of Johnson et al. (1981). When this phenomenon was first observed by Baase and Johnson (1979), these workers assumed that the effect was generated by an uncoupling of the changes in a number of helical parameters which are correlated with rotational strength of the positive band above 260 nm (Johnson et al., 1981). It is also possible, however, that this effect is due to an underlying phenomenon which governs $\Delta\Psi$ and thus affects $[\theta]_{275}$ through the latter variable's dependence on $\Delta\Psi$. This is very clear for the derivatives whose variations in the level of substitution, $R$, of the cationic amine decrease the value of $[\theta]_{275}$ and increase the winding angle.

The value of $[\theta]_{275}$ may also, of course, be affected directly by the influence of the cationic charge on the intrinsic electronic transitions of the base and by the perturbation of the hydration shell, $\Gamma$, in the minor groove due to its presence. We may express the corresponding changes in $[\theta]_{275}$ attributed to changes in these variables as

$$d[\theta] = (\partial[\theta]/\partial\Psi)_{R,\Gamma}d\Psi + (\partial[\theta]/\partial R)_{\Psi,\Gamma}dR + (\partial[\theta]/\partial\Gamma)_{\Psi,R}d\Gamma \quad (5)$$

The derivative data demonstrate that $\Psi$ and $R$ are functionally related and one suspects that $\Gamma$ and $R$ are also. From this standpoint, it would appear that the true independent variable for the experiments with the derivatives, at least, is $R$. Equation 5 above may thus be replaced by simpler form:

$$d[\theta]/d\Psi = (d[\theta]/dR)/(d\Psi/dR) \quad (6)$$

Experimentally, we have found that $d[\theta]/dR$ is approximately constant over the range of $R$ values (0–0.12) covered in these experiments. The nonlinear dependence of $[\theta]$ on $\Delta\Psi$ observed in Figure 5A implies a nonlinear dependence of $\Psi$ on $R$.

The question arises, therefore, as to the nature of the functional dependence of $\Psi$ on $R$. Its form is suggested by the commonality in the behavior of the underivatized samples in electrolyte solvents and the derivatives. Anderson and Bauer found that the winding angle changes of PM-2 DNA in electrolyte solvents were logarithmic functions of the salt concentration. Although their data were gathered in dilute salt solutions (0.05–0.3 M), these functions also predict, with reasonable accuracy, the values of $\Delta\Psi$ in the concentrated solutions of CsCl and $NH_4Cl$. Since $R$ is a measure of the electrolyte content of the minor groove, we might reasonably expect a linear relationship between log $R$ and $\Delta\Psi$ at least for the derivatives.

The logarithmic dependence of winding angle on the electrolyte content of the solvent was rationalized by Anderson and Bauer (1978) in terms of a generalized shielding effect on the electrostatic free energy of the DNA molecule. It could also arise, however, as a result of an underlying site-specific ion association process governed by the laws of mass action. Although the ion condensation theory of Manning (1978) has substantial experimental support (Bleam et al., 1983; Nordenskiold et al., 1984; Braunlin & Nordenskiold, 1984), there are a number of phenomena for which it cannot account, including the specific ion effects on CD (Hanlon et al., 1975) and NMR spectra (Bleam et al., 1980) and Anderson and Bauer's (1978) own winding angle data. In addition, more recent theoretical treatments of DNA's polyelectrolyte behavior which take into account the topography of the DNA duplex (Pack & Klein, 1984; Zimm & Le Bret, 1983; Matthew & Richards, 1984) indicate local concentrations of cations at the DNA surface higher than that predicted by the condensation theory and an asymmetric distribution in the grooves due to cation association at high-affinity sites between the negatively charged phosphates. If we make the assumption that the association can be described in multiple steps involving the maximal binding of $n$ cations per phosphate to DNA

$$DNA + nM^+ \rightarrow \rightarrow \rightarrow [DNA{\cdot}M^+_n]$$

with no change in the site binding affinity constant, $k$, then we can express the number of cations bound per mole of nucleotide, $R$, at any free cation concentration by the standard expression (Klotz, 1946):

$$R = nk[M^+]/(1 + k[M^+]) \quad (7)$$

$R$ in this expression is now the freely equilibrating cation content per mole of nucleotide. Rearrangement yields

$$\log [R/(n - R)] = \log [M^+] + \log k \quad (8)$$

The concentration of free cation is effectively the formal concentration (or activity) of the electrolyte since the DNA concentration is so low. If there are cooperativity effects with consequent variation in $k$ as the sites are filled, the dependence of log $[R/(n - R)]$ on log $[M^+]$ will exhibit a slope other than

1 but, within the range of values appropriate for this system, is still expected to be constant. Since the $\Delta\Psi$ data are linear functions of the log of the electrolyte content, then this analysis reveals that they are also linear functions of the log function above in eq 8. If the degree of association is sufficiently low, log $R$ alone should show linear dependence on $\Delta\Psi$.

The values of $R$ for the amine adducts can be assessed directly from the CD data. In order to obtain these values for the underivatized samples in electrolyte solvents, we have made the further assumption that the ellipticity changes of the underivatized samples in the salt solutions reflect equivalent numbers of bound cations. When the values of $R$ for the salt data are calculated in this manner and plotted together with the derivative data as log $R$ against $\Delta\Psi$, the results shown in Figure 5B are obtained. (Although our discussions have focused on $R$ as the independent variable, plotting the data in this manner facilitates the comparison with the data in panel 5A.) Within experimental error, the plot is linear with no significant difference observed between the derivative and the electrolyte data sets. The characteristics of this log $R$ relationship:

$$\log R = 1.60\Delta\Psi - 2.21 \quad (9)$$

have been used to generate the best-fitting exponential line through the data in Figure 5A. [Similar linear behavior was observed for the plots in which $n$ was set equal to values between 1 and 0.42 in the log function log $[R/(n - R)]$.]

This does not mean, however, that the alternative possibility of the appearance of a minor population of base pairs with an altered secondary structure at high values of $\Delta\Psi$ can be ruled out. In studies of calf thymus DNA, a third component whose shape resembles the CD spectrum of a distorted A form is required to account for the CD spectral changes in very concentrated electrolyte solutions (Hanlon et al., 1975). This spectrum is shown as the solid curve (3) in Figure 2B. It has subsequently been found to be predominant in high GC content DNAs in concentrated LiCl solutions (Ringquist, 1987). The spectra of the underivatized DNA samples in concentrated LiCl shown in panels B and C of Figure 1 exhibit distortions similar to those which arise when the component is present. The deviations are particularly obvious when the duplex structure is under superhelical stress. A spectral analysis of the number of independent spectral components present in the spectra displayed in Figure 1A,B, however, suggests the presence of similar distortions in the relaxed samples as well. The results revealed that, within our expected experimental error, the spectral data below 0.4° (or whose values of $[\theta]_{275}$ exceeded $5E + 3$ deg cm$^2$ dmol$^{-1}$) conformed, approximately, to a two-state transition whereas the entire set in the case of either the ccr samples in variable electrolyte solvents or the BuA derivatives required three or more independent components.

In an effort to extract the shape of this third component, we have performed a linear extrapolation of the spectral set observed in the dilute electrolyte solutions, where the transformation could be approximated by a two-component transition. The linear transformant has been extrapolated to a value of $[\theta]_{275}$ equal to that found for the observed spectrum of ccr DNA in 5.4 M $NH_4Cl$. The results of these efforts are shown as the solid curve (3) in Figure 2A. The difference between this extrapolated spectrum and the actual one observed in $NH_4Cl$ is shown as the dashed curve (2) in Figure 2B. The beaded curve (1) in this figure is the difference spectrum of the BuA derivative. The spectral distortions have the interesting property of having a minimal effect on the rotational strength of the positive band, as they are more or less symmetrical about 275 nm. The similar characteristics of all three spectra in this panel suggest a common origin.

In the case of the calf thymus DNA results, we had suggested that the component arose as a result of a conversion of a small fraction of base pairs to an A form at high levels of electrolyte and dehydration (Hanlon et al., 1975). In the absence of definitive data, however, one could equally well argue that it could be attributed to changes in the electronic characteristics of the base pairs due to changes in the environment of the grooves or direct ion association with the bases. In this present instance, the exaggerated appearance of the component in the spectrum of the supercoiled species would support the interpretation that the component is due to a change in secondary structure of a minor fraction of base pairs.

This minor fraction, however, does not contribute a winding change of a magnitude which exceeds the precision of the measurement (±0.05°). This is evidenced by the fact that the average winding angle changes in concentrated CsCl and $NH_4Cl$ can be calculated from the data of Anderson and Bauer (1978) obtained in dilute solutions of these electrolytes. From this, we may conclude that either the fractional amount of the component is very small or/and the winding angle of the structure is not radically different from that of the average of the majority of base pairs. Alternatively, the extent of formation of the species might also have a logarithmic dependence on the ion content of the grooves, as is the case for the majority of the base pairs. The precision of our measurements of both the winding angle changes and the spectral signals does not permit us to discriminate between these possibilities. What is certain, however, is that the appearance of this species cannot account for the nonlinear relationship between the rotational properties of the CD spectra and the winding angle changes.

## Conclusions

The common influence of the electrolyte content of the medium and variations in the level of amine substitution on the CD/winding angle relationship in DNA confirm our previous conclusions that the conformational properties of the amine derivatives are not exceptional and can be found in DNAs exposed to efficient electrostatic screening conditions. Since we know that the average winding angle changes for the derivatives are attributable to the placement of positive charges in the minor groove, it would seem reasonable to conclude that a similar concentration of localized ions, site bound in the minor groove to random-sequence DNAs in concentrated electrolyte solutions, is also responsible for the winding angle increases observed in those cases. The consequent decrease in the electrostatic free energy of the DNA molecule itself, due to the occupation of these sites, provides the energy for the mechanical transformation to a structure with a higher average winding angle. Furthermore, these results suggest, although do not prove, that the cation localization is specific for the G minor groove loci. If this is the case, then the average increase in winding angle probably rises as a result of some of the affected GC pairs, together with near neighbors, moving to a substantially higher winding angle rather than a uniform increase throughout the duplex. We would anticipate that the affected regions would have a more C-like structure with a deeper and narrower minor groove.

The observed exponential relationship between mean residue ellipticity and winding angle is unexpected, in view of the theoretical analysis of Johnson et al. (1981) which predicts a linear relationship over a very wide range of angular change. The latter analysis, however, was based on a model in which the environment of the helical grooves remained unchanged

as the conformational parameters changed. In the real world, the conformational transformations are driven by interactions of the DNA with solvent and ions of the environment. One might anticipate that the winding angle of the helix would be responsive, in a linear manner, to changes in the electrostatic free energy of the duplex due to environmental variations since the value of $\Delta\Psi$ is, to a first approximation, linearly related to the mechanical free energy change. When viewed in this light, the logarithmic relationship between the winding angle and the values of $R$ or the salt concentration of the solvent is understandable. The limiting slope of the $[\theta]_{275}$ vs $\Delta\Psi$ function (near $R = 0$), however, should reflect almost entirely the contribution of the first partial derivative, $(\partial[\theta]/\partial\Psi)_{R,\Gamma}$, in eq 5. The value arrived at by Johnson et al. (1981) for this function, linked to other secondary structural changes, was $-2.2E + 3$ deg cm$^2$ dmol$^{-1}$ deg$^{-1}$, which is actually very close to our limiting value of $-2.5E + 3$ deg cm$^2$ dmol$^{-1}$ deg$^{-1}$.

What is surprising, however, is the very wide range of salt concentrations over which the relationships derived by Anderson and Bauer (1978) accurately predict the winding angle changes for unmodified DNA. For associations governed by mass action, one would predict significant activity effects in the concentrated salt solutions. Yet the use of concentrations of electrolyte in the expressions of Anderson and Bauer (1978) will give values of the winding angle changes in 3.00 M CsCl and 5.4 M $NH_4Cl$ which agree very closely with that obtained experimentally by Baase and Johnson (1979) in those solvents. Values for 6.2 M LiCl, however, are significantly divergent, with the value predicted being 0.19° lower than the measured value. An examination of the behavior of the mean activity coefficients of these salts with concentration (Robinson & Stokes, 1959) suggests an explanation for these observations. The logarithmic relationships (log, log) between the activity coefficients and the salt concentration for CsCl and $NH_4Cl$ are approximately linear and have the same sign between 0 and 6 *m*. Thus, the slope terms in the expressions of Anderson and Bauer have incorporated the correction for the conversion of concentration to activity. In contrast, the relationship for LiCl is significantly nonlinear and requires two terms and a constant of opposite sign in the same concentration range. The sign and magnitude of the LiCl deviation for the calculated winding angle change are approximately what one would have expected, given the behavior of the activity coefficient changes below and above 0.3 M LiCl.

It is clear from these experiments that the correlation between the CD properties and the winding angle change is the same regardless of whether the CD effects are induced by increases in neutral electrolytes or by the attachment of variable amounts of simple amines. When the appropriate function is used for predictive purposes, the rotational strength of the positive band can thus be used as a reliable index of average changes in winding angle in the secondary structure, as long as B backbone geometry is maintained among the majority of the base pairs. Certain sequences, however, may be assuming radically altered conformations under the pressure of increased charge density in the grooves of the helix. Proteins which bind to DNA with the insertion of cationic charges in the helical grooves will thus influence helical periodicity. Although the average change may be small (e.g., 0.2 bp/turn), processes which demand a sequence periodicity can be significantly affected if the change is propagated over several turns of the helix.

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# Mechanism of ATP Inhibition of Mammalian Type I DNA Topoisomerase: DNA Binding, Cleavage, and Rejoining Are Insensitive to ATP†

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ABSTRACT: A general, unrefined mechanism of type I DNA topoisomerase action involves several steps including DNA binding, single-strand scission, strand passage, resealing, and, possibly, readoption of an active enzyme conformation. None of these steps requires an energy cofactor; however, we have shown previously that several mammalian type I topoisomerases are, in fact, inhibited by ATP. In this study, we wanted to examine which steps in the gross topoisomerase mechanism were sensitive or insensitive to ATP. Nitrocellulose filter binding experiments showed that ATP did not interfere with the binding of DNA by the enzyme and that ATP binding by topoisomerase was 5-fold greater in the presence of DNA than in its absence. Agarose gel electrophoresis in the presence or absence of ethidium bromide indicated that resealing was unaffected by added ATP. The addition of the adenine nucleotide did not alter the pattern of camptothecin-stimulated cleavage of DNA, indicating that strand scission was not the point of inhibition. To test whether strand passage or the readoption of an active conformation was an inhibited step, we used a unique DNA topoisomer as substrate. The results argued against readoption of an active enzyme conformation as an ATP-sensitive process.

DNA topoisomerases are enzymes that alter the topological state of DNA and thereby interconvert topological isomers via mechanisms that involve single-strand (type I enzyme) or double-strand (type II enzyme) breakage and rejoining. A large body of direct and indirect evidence has implicated the topoisomerases in almost every process involving DNA metabolism, including replication, transcription, repair, recombination, chromosome condensation, and viral packaging [reviewed by Wang (1985), Liu (1983), and Gellert (1981)]. Relatively little is known, however, about the regulation of topoisomerase activity. The expression of DNA gyrase seems to be regulated by the overall superhelicity of the bacterial chromosome; when the degree of supertwisting of the genome is low, DNA gyrase production is increased (Menzel & Gellert, 1983). This increase in gyrase is apparently necessary to counterbalance the relaxing efforts of the bacterial type I topoisomerase ($\omega$ protein). This topoisomerase interplay is further supported by genetic evidence. A number of bacterial mutants that have deleted the *topA* gene are able to survive due to compensatory mutations in *gyrA* or *gyrB* that reduce gyrase activity (DiNardo et al., 1982; Pruss et al., 1982).

Even less is known about the regulation of the eukaryotic topoisomerases. In vitro experiments have shown that histone H1 and several high mobility group (HMG)[1] proteins can stimulate topoisomerase I (Javaherian & Liu, 1983), while epidermal growth factor can stimulate topoisomerase II activity in human fibroblasts and Swiss/3T3 mouse fibroblasts (Miskimins et al., 1983). In addition, several modifications of topoisomerases lead to alterations of enzyme activity. For example, ADP-ribosylation inactivates (Ferro et al., 1983) and phosphorylation stimulates (Mills et al., 1982) topoisomerase I while phosphorylation of *Drosophila* topoisomerase II increases enzyme activity (Sander et al., 1984).

Although the type I topoisomerases do not require any energy source, we have recently reported an ATP effect which

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[1] Abbreviations: HMG, high mobility group; topo, topoisomerase; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; bp, base pair(s); SDS, sodium dodecyl sulfate.

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