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Circular dichroism and DNA secondary structure

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ABSTRACT

The change in average rotation of the DNA helix has been determined for the transfer from 0.05 M NaCl to 3.0 M CsCl, 6.2 M LiCl and 5.4 M NH_4Cl. This work, combined with data at lower salt from other laboratories, allows us to relate the intensity of the CD of DNA at 275 nm directly to the change in the number of base pairs per turn. The change in secondary structure for the transfer of DNA from 0.05 M NaCl (where it is presumably in the B-form) to high salt (where the characteristic CD has been interpreted as corresponding to C-form geometry) is found to be -0.22 (± 0.02) base pairs per turn. In the case of mononucleosomes, where the CD indicates the "C-form", the change in secondary structure (including temperature effects) would add -0.31 (± 0.03) turns about the histone core to the -1.25 turns estimated from work on SV40 chromatin. Accurate winding angles and molar extinction coefficients were determined for ethidium.

INTRODUCTION

X-ray diffraction patterns for DNA oriented in fibers and observed under certain conditions of salt and humidity define "A-form", "B-form" and "C-form" geometries (1, 2). The rationale for relating these geometries to CD spectra in solution comes from the work of Tunis-Schneider and Maestre (3), who measured the CD spectra of isotropic films under the same conditions that give the A-, B-, and C-forms in fibers. The characteristic conservative CD spectrum (4) was observed for films under the conditions which give the B-form diffraction pattern for fibers. A complete loss of intensity for the 275 nm CD band was observed for films under conditions which give the C-form diffraction pattern for fibers. Subsequently, a variety of changes in conditions (temperature, alcohol solvents, and high salt) have been found to cause this decrease in the 275 nm

CD band (5-12).

Certainly the film work (3), although strongly indicative of the relationship between fiber and solution conformations, is not definitive. The films must be isotropic for CD work, so that it is not possible to check them directly by recording the X-ray diffraction pattern. However, the assignment of the "C-form" CD spectrum is crucial since it is often found for DNA in condensed or packaged systems. CD studies of bacteriophage (13, 14), adenovirus (15), chromatin (16, 17), and nucleosomes (18, 19) all suggest that DNA adopts the structure represented by this spectrum under biological conditions.

Recent evidence suggests that DNA under the conditions which give the characteristic "C-form" CD spectrum cannot have the nine and one-third base pairs per turn which X-ray diffraction workers associate with the C-form found in fibers. Wide-angle X-ray patterns observed for DNA gels in 6 M LiCl differ little from the pattern observed for gels with low salt or from the pattern calculated for B-form DNA (20). The axial component of the CD of flow oriented DNA in 6 M LiCl is quite similar to the axial component of flow oriented DNA in low salt, suggesting that the conformations are similar (21). Raman studies indicate that the secondary structure of DNA in nucleosomes cannot be very different from the B-form (22, 23). Furthermore, the increase in helix winding angle on increasing Cs^+ concentration from 0.1 to 3 M is only 0.30° (24, 25) even though the 275 nm band decreases about 50% for this change in salt concentration (8, 26). Finally, CD spectra of DNA at low and high salt show virtually no difference except in the 275 nm band (27).

In this work, we relate the change in intensity of the 275 nm CD band to the change in the number of base pairs per turn (6), an important conformational parameter. Our results support the view that there is relatively little difference between the low and high salt conformations of DNA.

MATERIALS AND METHODS

Chemicals. Ethidium bromide, disodium ethylenediaminetetraacetic acid (EDTA), tris(hydroxymethyl)aminoethane (tris) and chloroquine diphosphate were purchased from Sigma. Optical grade

cesium chloride was purchased from Harshaw Chemical Co. All other salts were purchased from Baker. Agarose, standard low -m_r, was purchased from Bio-Rad.

Preparation of DNA. PM2 DNA was prepared according to standard procedures (28-30). PM2 samples with different numbers of superturns were prepared by incubation with topoisomerase activity from HeLa nuclei (31) in the presence of varying amounts of ethidium. Incubation was for at least twelve hours in 0.2 M Na^+, pH 7.5, at 20°C.

The topoisomerase activity was prepared by CsCl buoyant density centrifugation of chromatin from HeLa nuclei. The chromatin was made by the low salt method of Hancock (32). Density fractions at about 1.4 g/ml were a good source of the activity.

Binding of Ethidium by DNA. Our ethidium bromide, weighed after drying in vacuo, gave a molar absorptivity in water of 4758 at 460 nm and 5454 at 480 nm, in good agreement with the values of Waring (33). DNA concentrations were determined spectrophotometrically. The molar absorptivity of both calf thymus and PM2 DNA was taken to be 6600 at 260 nm (30). The binding of ethidium by DNA in both 5.4 M NH_4Cl, 10 mM tris-HCl, pH 8.2, and 6.2 M LiCl, 20 mM tris-HCl, pH 8.2, 1 mM Na_3EDTA was measured by the procedure of Waring (33). The molar absorptivity of bound dye at 460 nm was found to be 1543 using either calf thymus or PM2 DNA in 6.2 M LiCl, and 1431 using calf thymus DNA in 5.4 M NH_4Cl.

The molar absorptivity at 460 nm for free dye in both salts was a function of dye concentration in the range 0 to 125 μM. In 6.2 M LiCl, the apparent molar absorptivity of the free dye was fit to a line with slope -3.88×10^6 and an intercept of 5144. In 5.4 M NH_4Cl, the apparent molar absorptivity of the free dye was fit to a line with slope -3.11×10^6 and an intercept of 4761.

Sedimentation. Band sedimentation (34) was carried out as described by Wang (24, 35) using essentially identical equipment. No significant redistribution of ethidium was observed for the duration of each run. Boundary sedimentation was analyzed by the method of Van Holde and Weischet (36).

Gel Electrophoresis. A series of PM2 DNA samples were

relaxed using topoisomerase in the presence of 0 to 0.04 moles of ethidium per mole of DNA. The series used evenly spaced increments of 5.7×10^{-3} moles ethidium per mole of DNA. The agarose gel band counting method (37, 38) was used to determine linking number differences for these samples and the samples used for sedimentation runs.

Circular Dichroism Measurements. CD spectra were taken using a Durrum-Jasco J-10 recording spectrograph for wavelengths above 210 nm and a vacuum uv spectrograph (39) for measurements below 240 nm. Both instruments were calibrated using d-10-camphorsulfonic acid (Aldrich) using $\Delta\varepsilon = 2.37$ at 290.5 nm (40). All CD spectra were taken at 20°C.

Error Analysis. Binding data was fit by linear least squares. Standard deviations in the slope and intercept of the binding data were calculated from the scatter of the points. Sedimentation data were fit by a second order polynomial using data in the vicinity of the minimum. Standard deviations in the minima were calculated using an estimated ± 1% standard deviation in each sedimentation coefficient. This approach gives a larger standard deviation than is calculated from the scatter of the points. In the case of CsCl, the standard deviation of the equivalence point was taken as equal to that of the minimum. Errors from both the binding and sedimentation data were propagated to calculate the standard deviation in ν_c. The ν_c vs. ν_c and $\Delta\alpha$ vs. ν_c plots used the standard deviations of each point in determining the least squares fit and in determining the standard deviation of the slope and intercept. For the determination of linking number differences by gel electrophoresis, we estimate the standard deviation of the maximum of the gaussian distribution of superturns at ± 0.7 turns.

PROCEDURE

The basic idea behind our experiments is to take advantage of the topological constraint for covalently-closed, double-stranded DNA which imposes a "conservation of turns" (41, 42). This constraint means that the number of turns due to helix rotation, β, plus the number of superturns, τ, is equal to an integral constant, α, called the linking number.

Wang has made use of the conservation of turns for covalently-closed, double-stranded DNA to solve many conformational problems, including those induced by salt (24). Following his approach, the change in the average rotation per base pair in radians, $\Delta\theta$, for a change in secondary structure of the DNA helix caused by a transfer from ionic medium i to ionic medium j is given by

$$\Delta\theta = \theta_j - \theta_i = 4\pi(\tau_i - \tau_j)/N. \qquad (1)$$

where N is the number of nucleotides. It is important to emphasize that the change in the average rotation per base pair is the change in the rotation of the unstrained helix of DNA (DNA molecules containing no superturns). The average rotation is also temperature dependent (24, 43), but it is understood that all our data refer to 20°C.

The number of superturns can be found by removing all superturns with an unwinding agent (in this case the dye, ethidium) so that

$$\tau_i = (N\phi_i \nu_c^i)/2\pi \qquad (2)$$

where ϕ_i is the winding angle of the dye in medium i and ν_c^i is the number of dye molecules per nucleotide necessary to remove all superturns under these conditions. The titration of superturns can be followed by comparing the sedimentation rate of covalently-closed, double-stranded DNA with that of nicked DNA in the presence of varying amounts of dye.

The values of ν_c for ethidium bound to DNA can be found from the free dye concentration, c_f, at the sedimentation equivalence point by means of the binding equation

$$\nu/c_f = Kn - K\nu \qquad (3)$$

where K is the intrinsic binding constant to a site and n is the maximum number of dye molecules which can bind per nucleotide. Finally, bound and free dye can be measured spectroscopically since extinction coefficients for bound and free ethidium can be determined (33).

When covalently-closed, double-stranded DNA is relaxed in medium i before transfer to medium j, the change in τ can be measured directly by titration in medium j. We carried out this traditional experiment by using a sample which was relaxed in 0.05 M NaCl and the procedure outlined above to find $\Delta\theta$ for

the transfer to high salt. We also obtained additional data by using samples which were not relaxed in medium i. The difference in linking number between the various samples was determined by band counting on agarose gels (37, 38). Besides confirming the results of the traditional experiment, this additional data provided an absolute determination for the winding angle of ethidium.

RESULTS AND DISCUSSION

Titration of the Superturns. Values of K and Kn for the binding of ethidium to PM2 DNA (Equation 3) are given in Table I. The values for 3.0 M CsCl, 10 mM Na_3EDTA, pH 8.0, 20°C are from Wang (24) and have been adjusted to accepted values of the molar absorptivity.

The titration curves with ethidium which determine the number of superturns for various samples of covalently-closed, double-stranded PM2 DNA in the three salts studied are given in Figure 1. The five samples used are denoted A, B, C, D, and E in order of increasing number of superturns. For 6.2 M LiCl and 5.4 M NH_4Cl the sedimentation rate of nicked PM2 DNA is independent of ethidium concentration, so that the equivalence point is the same as the minimum. For 3.0 M CsCl the sedimentation rate of nicked PM2 DNA does depend on ethidium concentration (data not shown) and the equivalence point is slightly before the minimum. The equivalence points for these titrations are given in Table II.

Equation 3 is used to relate c_f of each equivalence point to ν_c for each salt condition. These results are given in Table III.

Band Counting on Agarose Gels. Figure 2 plots the change in

Table I: Binding Constants for Ethidium to PM2 DNA at 20°C

Salt	K	Kn
3.0 M CsCl (a)	2.77 (±0.16) x 10^4	0.587 (±0.020) x 10^4
6.2 M LiCl (b)	4.47 (±0.08) x 10^4	0.951 (±0.009) x 10^4
5.4 M NH_4Cl (b)	1.15 (±0.06) x 10^4	0.304 (±0.007) x 10^4

(a) Reference 24.
(b) This work.

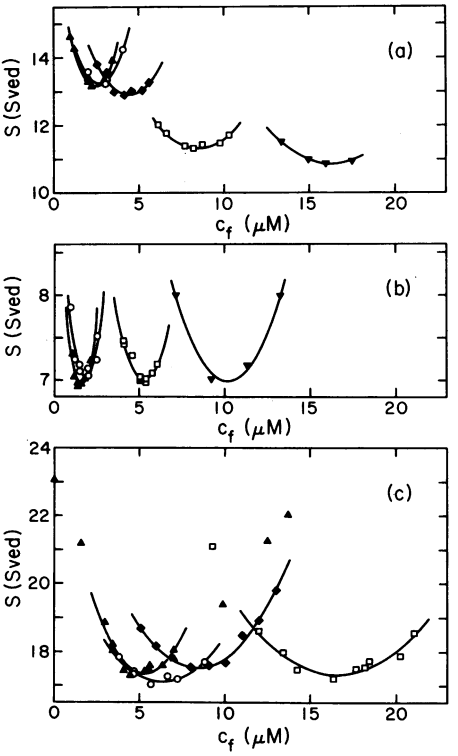

Figure 1. The uncorrected sedimentation coefficients of various samples of covalently-closed, double-stranded PM2 DNA are plotted against the free ethidium concentration in (a) 3.0 M CsCl, 10 mM Na_3EDTA, pH 8; (b) 6.2 M LiCl, 20mM tris-HCl, pH 8.2, 1 mM Na_3 EDTA; and (c) 5.4 M NH_4Cl, 10 mM tris-HCl, pH 8. Samples are "A", which is "native" PM2 DNA (▼), "B" (□), "C" (◆), "D" (○) and "E" (▲).

Table II: Ethidium Equivalence Points from Sedimentation

Sample	3.0 M CsCl Eq. Pt. (μM)	6.2 M LiCl Eq. Pt. (μM)	5.4 M NH_4Cl Eq. Pt. (μM)
A	15.60 ±0.37	10.23 ±0.08	----
B	8.10 ±0.14	5.23 ±0.09	16.54 ±0.18
C	4.30 ±0.12	----	8.38 ±0.13
D	2.65 ±0.10	1.92 ±0.03	6.40 ±0.20
E	2.40 ±0.04	1.66 ±0.04	5.35 ±0.09

linking number, $\Delta\alpha$, against the molar ratio of ethidium to nucleotide at the time of reaction with topoisomerase. The eight different samples were chosen so that, taken pairwise, they overlap on agarose gels and span the range of maximum sensitivity in $\Delta\tau$. Also shown by dotted arrows are the positions of samples A, B, C, and E from the titration studies with dye. The change in linking number for these samples are in Table III.

Ethidium Winding Angle. Since the linking number is a topological invariant, the $\Delta\alpha$ values determined for A, B, C, and E on gels are also correct for the high salt conditions. Furthermore, the average helix rotation per base pair is identical at each equivalence point for any given salt, so from the conservation of turns, $\Delta\alpha$ is equal to $\Delta\tau$. Thus equation 2 gives

$$\Delta\alpha = (N\phi/2\pi)\nu_c^K - (N\phi/2\pi)\nu_c^E \qquad (4)$$

where ν_c^K is the number of dye molecules per nucleotide necessary to remove all superturns from sample K in the salt considered, and ν_c^E is ν_c for sample E. The slope of this linear equation can be used to determine the ethidium winding angle, ϕ.

Figure 3 gives $\Delta\alpha$ as a function of ν_c for each of the three salts, using sample E as the zero point. From the least squares line for each salt, ϕ is -26.6 (±1.1)° for 3.0 M CsCl, -25.9 (±0.8)° for 6.2 M LiCl, and -26.2 (±1.5)° for 5.4 M NH_4Cl. These values do not differ significantly, and are in good agreement with Wang's value of -26 (±2.6)° in 3.0 M CsCl (24, 25).

Change in Average Rotation of the Helix. We use this data to calculate the change in the average rotation of the helix in two ways. First, using the intercept from Figure 3 to find values for ν_c^E averaged over all points, and Equation 2, we obtain the values in the third column of Table IV. An N of 19700 (30)

Table III: Experimental Results

Sample	3.0 M CsCl ν_c (x10^{-2})	6.2 M LiCl ν_c (x10^{-2})	5.4 M NH_4Cl ν_c (x10^{-2})	$\Delta\alpha$
A	6.39 ±0.20	6.68 ±0.07	----	-73.1 ±2.2
B	3.88 ±0.13	4.09 ±0.06	4.23 ±0.09	-38.1 ±1.7
C	2.26 ±0.10	----	2.33 ±0.06	-13.6 ±1.4
D	1.45 ±0.07	1.69 ±0.03	1.81 ±0.06	----
E	1.32 ±0.05	1.47 ±0.03	1.54 ±0.04	0.0 ±0.7

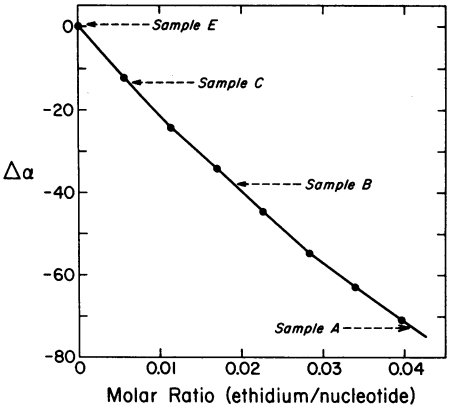

Figure 2. The change in the linking number, $\Delta\alpha$, for a series of eight PM2 samples is plotted against the molar ratio of ethidium to nucleotide present at the time of reaction with topoisomerase activity. The sample with no ethidium is taken as the zero point.

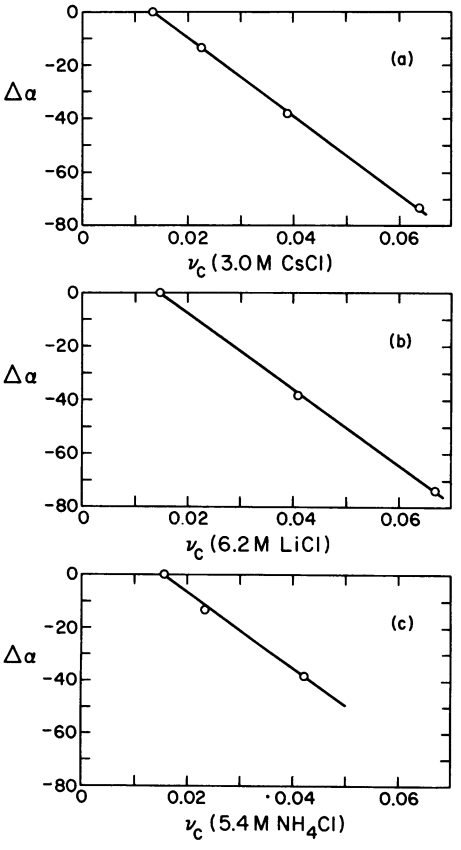

Figure 3. The change in the linking number, $\Delta\alpha$, is plotted against ν_c values for the PM2 samples in Table III. The least squares line for 3.0 M CsCl (a) is $\Delta\alpha = -1390\ (\pm 60)\ \nu_c + 19.2\ (\pm 1.5)$; for 6.2 M LiCl (b) is $\Delta\alpha = -1430\ (\pm 44)\ \nu_c + 20.7\ (\pm 1.2)$; for 5.4 M NH_4Cl (c) is $\Delta\alpha = -1412\ (\pm 83)\ \nu_c + 21.7\ (\pm 1.9)$. The length of PM2 DNA is taken to be 9850 base pairs (30).

Table IV: Comparison of CD and Change in Helix Rotation

Salt	Δε (275 nm)	Δθ (band counting)	Δθ (dye binding)
0.05 M NaCl	2.40	0.00 (a)	
0.20 M NaCl	2.17	0.11 (a)	
0.10 M CsCl	1.66	0.39 (a)	
0.30 M CsCl	1.47	0.53 (a)	
0.20 M NH_4Cl	1.47	0.57 (a)	
3.00 M CsCl	0.43	0.70 ±0.05	0.69 ±0.07 (b)
6.20 M LiCl	-0.35	0.76 ±0.04	0.77 ±0.08
5.40 M NH_4Cl	-0.57	0.79 ±0.07	0.81 ±0.09

(a) Data from reference 44. The standard deviation of these numbers was estimated at ±0.01° per base pair.
(b) 0.1 M to 3.0 M CsCl salt shift from reference 24.

and a ϕ of -26° (with an estimated error of ± 5%) were used in these calculations. To insure that sample E had, in fact, no residual superturns in 0.05 M NaCl, boundary sedimentation (36) was done in the presence of an equal amount of nicked DNA.

Second, we compare the ν_c values in the well studied salt, 3.0 M CsCl, to the ν_c values for 6.2 M LiCl and 5.4 M NH_4Cl in Figure 4. Rearranging Equations 1 and 2 in a straightforward manner gives the equation for a straight line

$$\nu_c^j = (\phi_i/\phi_j)\nu_c^i - \Delta\theta/2\phi_j \qquad (5)$$

The slopes in Figure 4 are nearly unity, confirming that ϕ is not significantly different in the three salts. The intercepts give Δθ (3.0 M CsCl to 6.2 M LiCl) = +0.08 (± 0.04)° per base pair and Δθ (3.0 M CsCl to 5.4 M NH_4Cl) = +0.12 (± 0.06)° per base pair. These values, combined with Anderson and Bauer's value of +0.39 (± 0.02)° per base pair for the transfer from 0.05 M NaCl to 0.10 M CsCl (44) and Wang's value of 0.30 (± 0.03)° per base pair for the transfer from 0.10 to 3.0 M CsCl (24, 25) give the values for the complete transfer found in the last column of Table IV.

These data appear quite reasonable. While we are aware of the dangers inherent in extrapolating Anderson and Bauer's linear plots of Δθ vs. log of ionic strength (44), we wish to point out that the values for 3.0 M CsCl, 6.2 M LiCl, and 5.4 M NH_4Cl do fall on such extrapolated lines within experimental error.

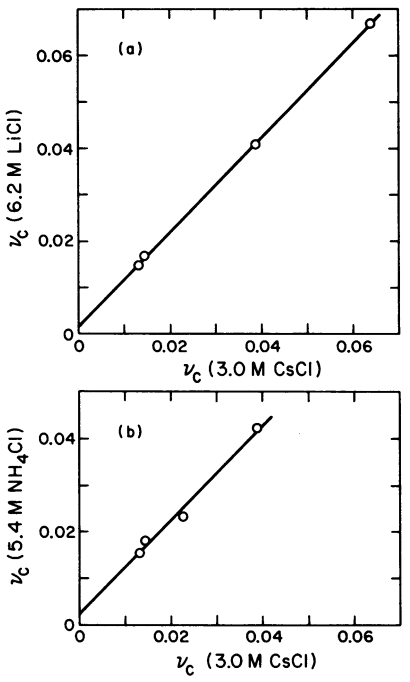

Figure 4. The ν_c values of PM2 samples "A" to "E" from Table III, in (a) 6.2 M LiCl and (b) 5.4 M NH_4Cl, are plotted against the ν_c values of these samples in 3.0 M CsCl. The least squares lines are: ν_c (6.2 M LiCl) = 1.019 (±0.036) ν_c (3.0 M CsCl) + 1.55 (±0.78) x 10^{-3}, and ν_c (5.4 M NH_4Cl) = 1.004 (±0.063) ν_c (3.0 M CsCl) + 2.4 (±1.2) x 10^{-3}.

Hinton and Bode (45) have used samples of covalently-closed, double-stranded λ DNA with various numbers of superturns to study the change in helix rotation accompanying shifts in NaCl concentration. Although their procedures are similar, their results disagree with ours. We have no explanation for this.

CD and Secondary Structure. CD spectra for the B-form to C-form transition of nicked PM2 DNA are given in Figures 5 and 6. Figure 5 presents CD spectra in 50 mM NaF (presumably the B-form), 0.75 M NH_4F (intermediate) and 6 M NH_4F (CD attributed to the C-form) measured into the vacuum uv to 174 nm. It is clear that the main effect of transfer to high salt is reduction of the 275 nm band. The intense 187 nm band, which we believe to be particularly sensitive to changes in base-base interactions (27), has changed very little. This indicates that these two forms have about the same number of base pairs per turn. The loss of intensity in the 275 nm band could be due to changes in base-sugar interactions.

Figure 6 shows the collapse of the 275 nm band for a vari-

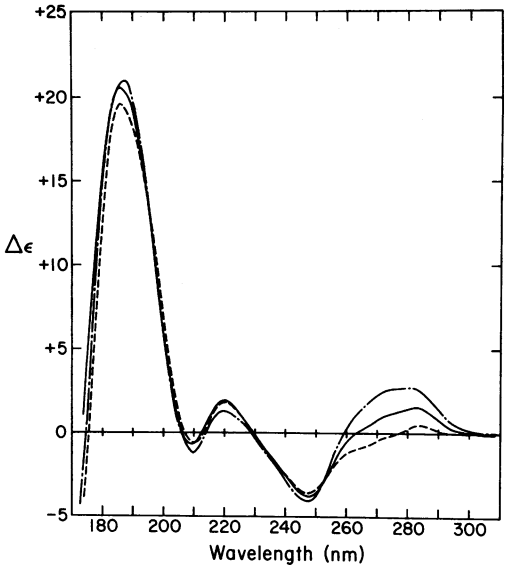

Figure 5. The CD of nicked PM2 DNA in 0.05 M NaF (— · —); 0.75 M NH_4F (————); and 6.0 M NH_4F (- - - -) at 20°C.

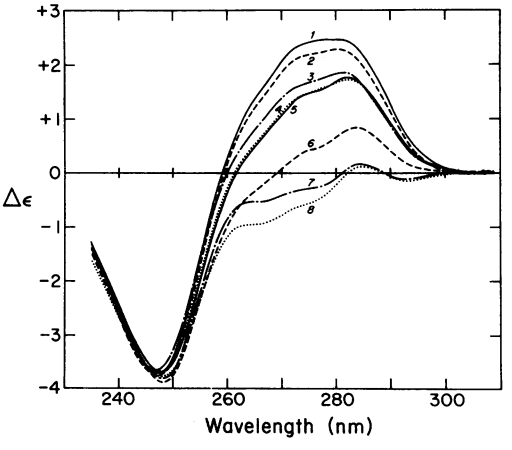

Figure 6. The CD of nicked PM2 DNA at 20°C in 0.05 M NaCl (1); 0.2 M NaCl (2); 0.1 M CsCl (3); 0.3 M CsCl (4); 0.2 M NH_4Cl (5); 3.0 M CsCl (6); 6.2 M LiCl (7); and 5.4 M NH_4Cl (8). The buffer was 1 mM $NaPO_4$, pH 8, in all cases.

ety of salts. In addition to our $\Delta\theta$ data for high salt, Anderson and Bauer (44) have used the agarose gel, band counting method to follow changes in $\Delta\theta$ for PM2 in the more limited salt range of 0.05 M to 0.3 M. We have added widely spaced data points selected from their work to the high salt data and have taken the directly comparable CD spectra shown in Figure 6. This area of the spectrum is well studied for DNA and the salt induced reduction of these bands has been interpreted as a gradual transition from B to C geometry (5-12). The $\Delta\theta$ data is in the correct direction for this interpretation, since we find the addition of salt to increase the helix rotation per base pair (winds the helix more tightly). However, the magnitude of this effect is considerably less than expected. The change in $\Delta\theta$ on transfer from 0.05 M NaCl ("B-form" DNA) to 5.4 M NH_4Cl ("C-form" DNA) is only +0.8°. Assuming B-form DNA to have 10 base pairs per turn, this would place C-form DNA at 9.8 base pairs per turn. While it is clear that there is latitude in the X-ray diffraction numbers, C-form is thought to be between 9.3 and 9.6 base pairs per turn (2, 46).

We compare $\Delta\varepsilon$ at 275 nm, the point of maximal difference in the first CD band (47), with $\Delta\theta$ for these salts in Table IV and Figure 7. With these results it is possible to relate the CD directly to the change in the number of base pairs per turn.

The CD of DNA arises from the chromophoric bases interacting with the sugar-phosphate groups and interacting with each other. These interactions, and thus the resulting CD, depend on the secondary structure of the DNA which can be modified by (a) changes in the average rotation per base pair, (b) changes in the relative position of the bases for a given average rotation, and (c) changes in the position of the bases relative to the sugar-phosphate groups. Our experiments with covalently-closed, double-stranded DNA only follow changes in the average rotation per base pair.

Figure 7 indicates that, among other changes in secondary structure resulting from ionic shifts at low salt, there is a change in average rotation of about 0.5° per base pair when the CD changes from a $\Delta\varepsilon$ (275) of +2.4 to +1.5. However, a larger change in $\Delta\varepsilon$ (275) from +1.5 to -0.6 shows a concomitant change

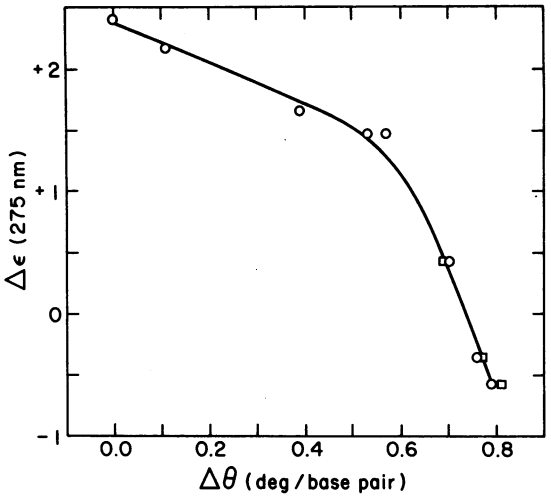

Figure 7. The Δε values at 275 nm for PM2 DNA in various salts are plotted against the change in average rotation of the helix, Δθ, for the transfer of DNA from 0.05 M NaCl to higher salt.

in average rotation per base pair of less than 0.3°. Although there is no reason to believe that the CD should be linearly related to Δθ, the break at Δε (275) equals +1.5 apparently indicates that other changes in the secondary are gaining in importance at higher salt.

Nucleosomes. Since few proteins have significant CD intensity above 240 nm, it should be possible to use CD data at 275 nm and Figure 7 to determine changes in nucleic acid secondary structure in protein-DNA complexes. For the particular case of nucleosomal DNA, both CD data (47) and linking number measurements (37,48, 49) are available.

Crick (50) points out that the expected change in linking number for nucleosomes is a function of the exact space path of the DNA ribbon, and that abrupt and unexpected discontinuities in linking number can result from certain non-simple arrangements. However, assuming that the space path of the axis of the DNA ribbon is a simple left-handed helix around the core protein (51), we can do the following calculations for the number of turns of DNA around a nucleosome core.

Shure and Vinograd (48) have estimated the change in linking

number corresponding to the assembly of the mature SV40 particle (0.2 M NaCl at 37°C) to be -1.25 turns per nucleosome. Since the work of Young and Champoux (52) suggest that the internucleosomal DNA of the SV40 minichromosome is under no topological constraint, we assume that this change in linking number is also valid for the nucleosome.

Calculation of the change in secondary structure between the DNA of a nucleosome and DNA in 0.2 M NaCl at 37°C requires correction for temperature effects, which have been found to be independent of salt effects (13, 24, 43). The change due to temperature is -0.011° per base pair per degree C, so the total change for 17°C is -0.19°. Thus, relative to 0.05 M NaCl at 20°C, the net $\Delta\theta$ for 0.2 M NaCl at 37°C is -0.077°. The DNA of a 143 base pair nucleosome in 0.25 mM EDTA has a $\Delta\varepsilon$ of 0.24 (47) which corresponds to $\Delta\theta$ = +0.71°. No corrections are necessary here since $\Delta\theta$ is relatively insensitive to $\Delta\varepsilon$ at this position in Figure 7. For removal of the core protein we have a change in average helix rotation of -0.79 per base pair. The total change for 143 base pairs is then -113° or -0.31 (± 0.03) secondary turns. Since the change in linking number per nucleosome is -1.25, the DNA ribbon is wrapped around the core about -1.6 turns.

It is also interesting to make a simple geometrical calculation on the nucleosome. Pardon *et al* (53) report 110A for the diameter of the nucleosome and 25A for the diameter of the DNA wrapped around the core. Griffith (54) reports a rise of 2.9A per base pair for double-stranded DNA prepared for electron microscopy using several different procedures. Assuming that the DNA which wraps around a core has an average rise of 2.9A at the center of the helix, one computes about 1.6 turns around the core for a 143 base pair nucleosome.

CONCLUSIONS

1. The total change in the average rotation of the DNA helix is found to be +0.8° per base pair with an error of ± 10% for the transfer from 0.05 M NaCl to 5.4 M NH_4Cl.
2. The change in secondary structure for the transfer of DNA from 0.05 M NaCl (where it is presumably in the B-form) to

5.4 M NH_4Cl (where it exhibits a CD spectrum which has been attributed to the C-form) is found to be -0.22 (± 0.02) base pairs per turn.

3. The small change in the intensity of the 187 nm CD band for the change in salt from 0.05 M NaF to 6.0 M NH_4F (Figure 5) also indicates a small change in the number of base pairs per turn.
4. Figure 7 shows how the intensity of the CD of DNA at 275 nm can be related directly to the change in the average rotation of the helix.
5. With Figure 7 and other data (24, 43, 47) it is now possible to compute the change in the number of base pairs per turn of DNA for the removal of the protein from mononucleosomes. For the conditions used by Shure and Vinograd (48) the result is -0.31 (± 0.03) turns.
6. This change in secondary structure of -0.31 turns for the DNA wrapped about a histone core can be combined with the change in linking number of -1.25 turns per nucleosome estimated for the removal of the protein cores from a mature SV40 particle (37, 48) to give about -1.6 turns about the histone core, or about 90 base pairs per turn.
7. The ethidium winding angle in the three salts studied was determined by band counting on agarose gels to be -26° with an error of ± 5%.
8. The molar absorptivity for free ethidium monomer at 460 nm was found to be 4758 in aqueous solution and in 3.0 M CsCl, 5144 in 6.2 M LiCl, and 4761 in 5.4 M NH_4Cl. The molar absorptivity at 460 nm for ethidium bound to DNA was found to be 1482 for 3.0 M CsCl using calf thymus DNA, 1543 for 6.2 M LiCl using either calf thymus or PM2 DNA, and 1431 for 5.4 M NH_4Cl using calf thymus DNA.

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