Correlations between the Duplex Winding Angle and the Circular Dichroism Spectrum of Calf Thymus DNA†

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ABSTRACT: Circular dichroism spectra have been obtained on linear calf thymus DNA under conditions where information on the effect of these conditions on the duplex winding angle of circular PM-2 DNA is available from the studies of others [Anderson, P., & Bauer, W. (1978) *Biochemistry 17*, 594]. Our experiments reveal that the magnitude of the positive band above 260 nm decreases in a linear manner as the duplex winding angle increases, as a result of decreases in temperature and/or increases in the content and variations in the nature of the cation concentration of the aqueous medium. The character of the changes in the spectral properties as well as the direction of the changes in the winding angle is consistent with the interpretation that DNA moves along a conformational continuum, from a more B-like to a more C-like state as the temperature decreases or the cation concentration increases. Temperature appears to have an additional discriminating effect on the relative weights which the GC and the AT pairs make to the spectral properties; the contributions of the latter appear to decrease with increasing temperature. These results support our previous conclusion that the specific cation effect on the circular dichroism spectrum of DNA is a real conformational effect rather than a perturbation of the spectral properties of the base chromophores [Hanlon, S., Brudno, S., Wu, T. T., & Wolf, B. (1975) *Biochemistry 14*, 1648].

A variety of studies with relaxed and supercoiled procaryotic DNAs has established the fact that the winding angle, ψ, of the DNA duplex is a function of temperature and the nature and concentration of the ions of the aqueous solvent in which the DNA is dissolved. In general, ψ increases with decreasing temperature and increasing ionic strength. Furthermore, there is a specific cation effect, with some ions being more efficient than others, in effecting the change in ψ (Wang, 1969, 1974; Depew & Wang, 1975; Hinton & Bode, 1975; Shure & Vinograd, 1976; Anderson & Bauer, 1978). Equally extensive are the studies on the effects of temperature and ionic content of the medium on the circular dichroism (CD)[1] spectra of eucaryotic and procaryotic DNAs in the linear, relaxed, and supercoiled forms. These studies have shown that the positive band above 260 nm in the CD spectrum increases with increasing temperature but can be dramatically decreased, and even assume negative values, with decreasing temperature and/or increasing ionic strength. Again, there is a difference in efficiency Maestre, various cations of the same charge type (Tunis-Schneider & Maestre, 1970; Campbell & Lochhead, 1971; Maestre & Wang, 1971; Studdert et al., 1972; Gennis & Cantor, 1972; Ivanov et al., 1973; Hanlon et al., 1975, 1978; Brahms et al., 1976; Wolf et al., 1977). Although it would seem logical that these two phenomena, changes in the winding angle and changes in the CD spectrum, are related through the common effects of ions and temperature, the absence of systematic data on the dependence of ψ on the ionic content of the medium has frustrated attempts at illuminating the quantitative relationship between the CD properties and ψ, although some effort has been made in that direction (Ivanov et al., 1973).

The recent publication, however, of an extensive study of the effects of cations on the change in the value of ψ for PM-2 by Anderson & Bauer (1978) has provided a wealth of data which makes such a study feasible. More specifically, these authors have evaluated the change in ψ which results when PM-2 DNA, relaxed and subsequently closed (by the N/C enzyme) at 20 °C in a given salt solution, is transferred to a given reference solvent, equivalent to an ionic strength of ca. 0.04, at the same temperature. These authors have found that ψ of this DNA increases as the concentration of electrolyte increases. There is also, as previously mentioned, a specific cation effect such that the order of efficiency in promoting this increase at constant concentration of electrolyte is $NH_4^+ > Cs^+ > Rb^+ > Li^+ > K^+ > Na^+$ for the chloride salts.

We were particularly struck with this order as it corresponds to the order of effectiveness of these ions in depressing the positive band of the CD spectrum of calf thymus DNA at 27 °C (Hanlon et al., 1975). Since the overall composition of PM-2 DNA (42% GC content; Espejo, 1969) is equivalent to that of calf thymus DNA, we felt that it was feasible to utilize the results of Anderson & Bauer (1978) and Depew & Wang (1975) for the salt and temperature dependence of ψ in order to explore the correlation between the CD spectral properties and ψ. We were specifically interested in determining (1) whether the thermally induced transitions in the CD spectrum of DNA belonged to the same family and contained the same spectral components as those transitions induced by changes in the concentration and nature of the cations in the medium and (2) whether both of these phenomena related in a similar manner to changes in ψ induced by these two experimental variables. This paper reports the results of these studies.

Experimental Section

A commercial preparation of calf thymus DNA, lot 900007 from Calbiochem, was used in these experiments. The properties of this sample have been described in a previous publication (Hanlon et al., 1976). Its protein content was less than 1%, as determined by the Lowry method, and its weight-average molecular weight was 8.6×10^6, as determined

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[1] The abbreviation CD is employed for circular dichroism in the text, tables, and legends for the figures of this work.

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from its sedimentation coefficient. Its spectral behavior was comparable in all respects to the calf thymus DNA sample, Sigma lot 802184, which has been extensively characterized by our laboratory (Johnson et al., 1972; Hanlon et al., 1975; Wolf & Hanlon, 1975; Wolf et al., 1977).

Stock solutions of this DNA at a concentration of ca. 1 mg/mL were prepared in 0.05 M concentrations of NaCl, KCl, LiCl, CsCl, and NH_4Cl adjusted to pH 7.0 by very small additions of NaOH or HCl. Dilute solutions of DNA at a concentration appropriate for CD spectroscopy (2.4×10^{-4} mol of nucleotide residues/L) were prepared at three salt concentrations between 0.05 and 0.30 M from the appropriate DNA stock solution and stock concentrations of each of the electrolytes listed above. One set of these solutions contained 2 mM Tris buffer at pH 7.0; a duplicate set prepared from a separate set of stock solutions was simply adjusted to pH 7 by the addition of small amounts of NaOH. The CD and absorption spectral properties of these solutions, referred to as the "salt series" in subsequent discussions, were examined at a fixed temperature. No sensible differences were observed in the spectral properties of the solution which contained Tris buffer compared to its pH-adjusted duplicate.

An additional set of dilute DNA solutions in 0.02, 0.05, and 0.10 M NaCl, adjusted to pH 7 by the addition of NaOH, was also prepared from a DNA solution in 0.05 M NaCl. Aliquots of each were examined in separate experiments in which the absorption properties and the CD properties were examined as a function of temperature. This set of solutions is referred to as the "premelting series".

The concentration of DNA in each dilute solution was determined from the absorption spectrum taken with a Cary 14 recording spectrophotometer at room temperature (ca. 21 °C) in 1-cm quartz cells. An extinction coefficient of 6600 M^{-1} cm^{-1} was used to convert the absorbance at 259 nm to concentration on a mole of nucleotide per liter basis.

Thermal melting profiles of the DNA solutions employed in the premelting experiments were obtained with a Cary 15 recording spectrophotometer equipped with a jacketed cell holder and circulating water bath. Temperatures of the cell contents were measured with a Yellowsprings bridge and thermistor assembly, with the thermistor probe inserted directly in the fluid at the top of the cell. Only a partial profile for the sample in 0.10 M NaCl could be obtained because of the high temperatures required to melt out the DNA at this ionic strength. An estimate of T_m for this latter sample based on the observed hyperchromic increase of the other two solutions in similar experiments (0.02 and 0.05 M NaCl) is ca. 81 °C, which is a value consistent with what one would expect at this ionic strength for the GC content of calf thymus DNA (Marmur & Doty, 1959; Gratzer et al., 1970). The T_m's for the lower salt concentrations were 76.5 °C for the 0.05 M NaCl solution and 70.0 °C for 0.02 M NaCl. The hyperchromic increase in the absorbance at the maximum upon complete melting was 1.44 times that at 20 °C. The hyperchromic increase occurring up to the toe of the transition (presumably reflecting the melting of very short segments or single strand ends) amounted to no more than 4% of the total hyperchromic increment of 0.44.

Circular dichroism spectra were obtained with two instruments, a Jasco Model J 40A and a Cary Model 60, equipped with a 6001 CD attachment. The calibration of both instruments was checked with a sample of *d*-camphorsulfonic acid, purified by sublimation from an Eastman product. A solution of this reference compound was prepared at a concentration of 1.3609 mg/mL and the ellipticity was measured at 290.5 nm in a 1-cm cell, first in the Cary 60 and then in the J-40A. The response of both instruments was essentially identical, within experimental error, and yielded the anticipated magnitude of the signal (0.4192°) (Cary 60 6001 CD Instruction Manual) within ±1%.

The comparable behavior of the two instruments was also checked by running the same solution of DNA in the same cell on the two machines. After correcting for the temperature difference in the cell chambers (see below), the two spectra obtained on the same solution were usually observed to be indistinguishable when compared in terms of their mean residue ellipticities. When discrepancies were observed, they could generally be attributed to base-line problems and the appreciably higher noise level of the Cary 60 instrument.

All of the premelting experiments as well as one set of the salt series were conducted with the J-40A. The salt series solutions were examined with the instrument equilibrated at 21.5 ± 0.5 °C in a constant temperature room, as determined by measuring the temperature of the cell holder with the Yellowsprings bridge and thermistor assembly.

The premelting experiments utilized a jacketed cell thermostated with a circulating bath. Temperature of these latter experiments ranged from 4 to 21 °C for the 0.05 M solutions, 28.5 to 50.5 °C for the 0.02 M NaCl solution, and 27 to 66 °C for the 0.10 M NaCl solution. Since the thermistor probe could not fit into the neck of the cell, temperature of the cell itself was measured by taping the flat portion of the probe to the jacketed surface of the cell.

The CD spectra of an additional set of salt series solutions, as well as spectra of the salt series set employed in the experiments using the J-40A, were determined with the Cary 60 CD at a temperature of 25.5 °C,[2] as measured with the Yellowsprings bridge and thermistor. The spectral data obtained on this spectropolarimeter showed consistently more scatter compared to the J-40 data, although the slopes and intercepts obtained from separate regression analyses of the data from the two instruments did not differ significantly.

In our experiments, we have defined $\Delta\psi$ as the average change in the helix winding angle in going from a given reference state to some other state. For the salt series, these values were obtained from Figure 5 and Table I of Anderson & Bauer (1978). It should be noted that these authors defined $\Delta\psi$ in a reverse manner—that is, as the average change in the duplex winding angle in going from a given salt solution to a standard reference solution. Thus, the values of $\Delta\psi$ presented in our data plots and employed in our regression analyses have been multiplied by −1. The 0 value of $\Delta\psi$ (corresponding to the value of ψ in the reference state) in the work of Anderson and Bauer was that value appropriate for an electrophoresis buffer, pH 8, at 20 °C with an ionic strength and salt effect equivalent to ca. 0.04 M NaCl. We found it more convenient to use 0.05 M NaCl (±2 mM Tris), pH 7, at 21.5 °C as our zero reference point. The calculated difference in $\Delta\psi$ between these two sets of conditions is negligible since the slight difference in temperature induces a change which is about equal but of opposite sign to the change induced by the small difference in NaCl concentration.

The changes in the value of $\Delta\psi$ induced by changes in temperature were calculated using an average coefficient of -1.1×10^{-2} deg/°C for $(d\psi/dT)$. This was the value reported by Anderson & Bauer (1978) for the NaCl solution in the

[2] Although normally the Cary 60 chamber is thermostated at 27 °C, the malfunctioning of the thermostat of the circulating bath coupled with heat losses to the room resulted in chamber temperatures which ranged between 25 and 26 °C. We have averaged this as 25.5 °C.

temperature range of 4 to 20 °C. This corresponded reasonably well to the average of the corrected values reported by Wang (1969) and DePew & Wang (1975) (-1.0×10^{-2} to 1.2×10^{-2} deg/°C) for a broader thermal range (0 to 42 °C) for a variety of DNAs. Values of $\Delta\psi$ for the premelting experiments were calculated relative to 21.5 °C and displaced according to the salt content of the medium. This displacement amounted to −0.04 °C for the 0.02 M NaCl solution and +0.06 °C for the 0.10 M NaCl solution. The values of $\Delta\psi$ for those spectra in the salt series obtained in the Cary 60 at 25.5 °C were also corrected to 21.5 °C by subtracting a constant increment of 0.044 °C.

Analysis of the number of independent components contributing to the observed changes in a given set of spectra utilized the "LMS Component Analysis" described in a previous publication (Wolf et al., 1977). This procedure attempts to analyze a spectral series in terms of a linear combination of members of either the same series or a different series. That is, at any wavelength, λ_j, one may express the observed signal of the ith solution, $[\theta]_{\lambda j}^{i}$, as

$$[\theta]_{\lambda j}^{i} = \sum_{q} f_q [\theta]_{\lambda j}^{q \neq i}$$

where $[\theta]_{\lambda j}^{q}$ are the observed signals of the q members of the series. Using a multivariable linear regression analysis which does not incorporate an intercept (method I of Hanlon et al., 1975), the best fitting coefficients, f_q, of the linear combination may be evaluated without any assumptions as to sign, magnitude, or dependencies. It can be shown that the minimum number of basis spectra required to bring the $\sum f_q$ to 1 ± 0.05 (where the 0.05 represents our estimate of the effects of experimental error) with a standard deviation of the fitted curves (SD) of reasonable magnitude ($\pm 0.15 \times 10^3$ deg cm^2/dmol for our experimental conditions) corresponds to the number of independent spectral components whose variations in relative amounts give rise to the observed spectral changes. Commonality of the components present in two separate sets of spectra may be tested by interchanging the basis spectra of one set for the other. If the value of $\overline{\sum f_q}$ remains at 1 with a reasonable SD, then one may conclude that all of the components present in one set are also present in the other.

In practice, one first selects two spectra to serve as the basis set for the analysis of the remaining members, i, of the solution series. If the mean average value of the sum for the entire series (excluding the q spectra used as the basis set), $\overline{\sum f_q}$, is 1.0 with a given acceptable mean average standard deviation, $\overline{SD}$, the series is reanalyzed with an added basis spectrum. If $\overline{\sum f_q}$ remains 1.0 and the $\overline{SD}$ is unchanged, then one may conclude that the given series of spectra can be accounted for adequately in terms of a linear combination of two components.

If, conversely, the value of $\overline{\sum f_q}$ is greater or less than 1, with a high $\overline{SD}$ for the first trial using two basis spectra, then additional spectra are added to the basis set until the value of $\overline{\sum f_q}$ becomes 1. The point at which the minimum number of basis spectra required for this has been added is usually evident by a dramatic decrease in $\overline{SD}$. If two series of spectra do not share common components, the exchange of their basis spectra will yield values of $\overline{\sum f_q} \neq 1$ with high values of $\overline{SD}$, and adding more basis spectra will not alleviate this situation. If the two series share common components, however, the values of the $\overline{SD}$ upon exchanging basis spectra are apt to remain reasonable even though the sums, $\overline{\sum f_q}$, are not equal to 1.

This method of component analysis will not, of course, detect two or more components which, however different in spectral characteristics, transform in a linked—or dependent—fashion, nor will it detect simple wavelength shifts of a given spectral band if the shift is contained within the range of wavelengths analyzed.

Table I: Component Analysis of DNA Spectra

data set	basis set	no. of basis spectra	$\overline{SD}$[a]	$\overline{\Sigma f}$[a]	no. of independent spectral component
salt	salt	2	0.116	1.016	2
salt	salt	3	0.105	1.000	2
salt	premelting	2	0.250	0.802	spectra not totally interchangeable
salt	premelting	3	0.123	0.880	
salt	premelting	4	0.115	0.897	
premelting	premelting	2	0.277	0.956	>2
premelting	premelting	3	0.148	0.998	3
premelting	salt	2	0.303	1.184	spectra not totally interchangeable
premelting	salt	3	0.285	1.119	

[a] $\overline{SD}$ ($\times 10^{-3}$ deg cm^2/dmol) and $\overline{\Sigma f}$ are the mean average of the standard deviations and the sum of the coefficients, respectively, obtained when the individual spectra were filled with the basis set.

The multivariable regression analysis used for the component analysis described above utilized an IBM 370/168 computer. The simple linear regression used in calculating the dependence of $[\theta]_{\lambda j}^{OBS}$ on $\Delta\psi$ employed a programmable Monroe Calculator Model 1766. Tests of significance included Student's t test and a covariance analysis using the F test (Snedecor & Cochran, 1967). On the basis of these tests, the linear regression characteristics reported in Table II were deemed significantly different from one another, when indicated as such in the text.

Results and Discussion

The effects of temperature and salt content of the aqueous medium on the CD spectrum of DNA are shown in Figure 1A,B. Figure 1A shows the spectra in 0.10 M concentrations of NaCl, KCl, LiCl, CsCl, and NH_4Cl at a constant temperature (21.5 °C). Panel B displays the spectra obtained as a function of temperature at various concentrations of NaCl (0.02, 0.05, and 0.10 M). The major effect to note is the depression or enhancement of the positive band between 260 and 300 nm without an accompanying effect on the negative band between 230 and 260 nm.

Although these two sets of spectra appear to belong to the same family, a careful inspection reveals certain minor differences in the shapes. This qualitative observation is confirmed by the LMS component analysis, the results of which are given in Table I. The set of spectra at constant temperature, perturbed by changes in salt content (the salt series), is adequately accounted for in terms of a two-component transition; increasing the number of basis spectra to three results in no marked improvement in $\overline{\sum f_q}$ or the $\overline{SD}$. In contrast, the set of spectra obtained as a function of temperature (the premelting series) needs an additional component to fully account for all of the spectral signal of the positive band. The magnitude of this component, however, is small since even two basis spectra can, on the average, account for 96% of the signal of any other spectrum. Members of each series, however, are not totally interchangeable, insofar as two or more members of the premelting series cannot account fully for the changes in the salt series and vice versa. Variation in temperature has apparently uncoupled two spectral components which were linked as one in the salt series and/or added an additional component. The additional component could not be assigned to denatured or fully unstacked DNA since

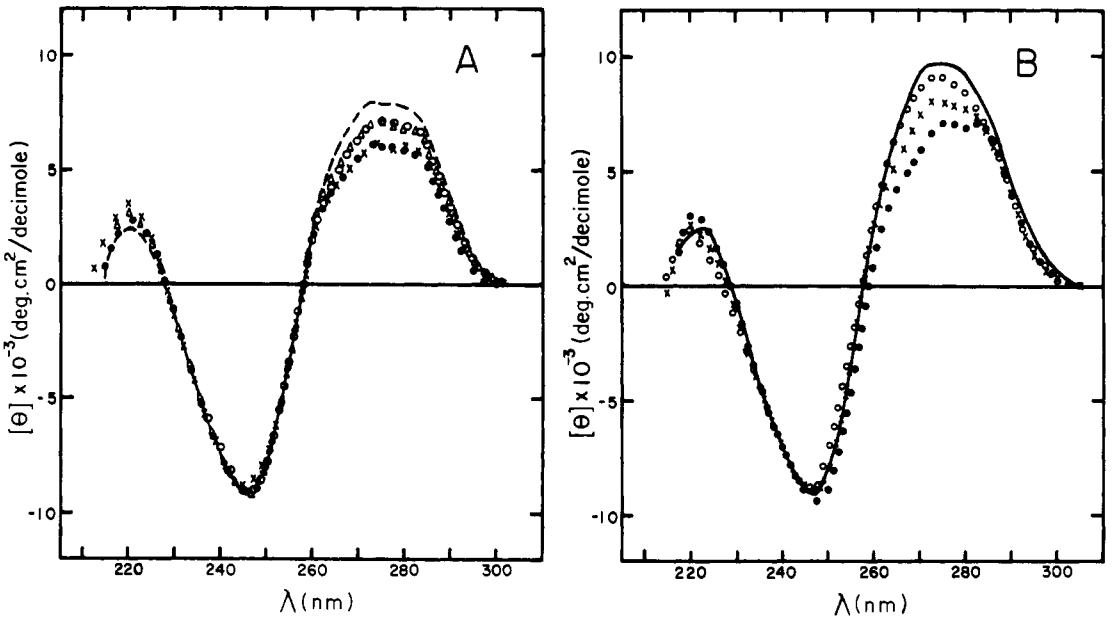

FIGURE 1: Effects of salt and temperature on the circular dichroism spectra of calf thymus DNA. (A) CD spectra at 21.5 °C, pH 7, in 0.10 M concentrations of NaCl (---), KCl (○), LiCl (△), CsCl (●), and NH_4Cl (×); (B) CD spectra at 66 °C in 0.10 M NaCl (—), 50.5 °C in 0.02 M NaCl (○), 26.3 °C in 0.05 M NaCl (×), and 6 °C in 0.05 M NaCl (●).

the thermal melting profiles of the absorption spectra indicated that all of the premelting spectra were obtained at temperatures equal to or greater than 15 °C below the T_m at the given ionic strength. Furthermore, the small amount of uncooperative single strand melting which one always sees in sheared commercial DNA amounted to no more than 4% of the total hyperchromic change.

The correlation between these spectral effects and the change in the value of ψ is shown in Figure 2A–D. In the upper portion of this figure (2A and 2B), we have plotted the behavior of the mean residue ellipticity, $[\theta]$, at a wavelength conveniently near the maximum (275 nm) and the minimum (245 nm) of the positive and the negative band, respectively. In the lower part we have plotted the values of $\int([\theta]_\lambda/\lambda)\,d\lambda$, a function which is proportional to the rotational strength of the positive (260–300 nm) and the negative (230–260 nm) band. The reference value of 0 on the abcissa was taken as 0.05 M NaCl at 21.5 °C, for reasons described in the Experimental Section. The positive values of $\Delta\psi$ reflect those values appropriate for the salt data and those premelting spectra obtained below 21.5 °C, while the negative values are those relevant to the premelting spectra above 21.5 °C. The values of $\Delta\psi$ for the premelting data were calculated relative to 21.5 °C, using an average thermal coefficient, $d\psi/dT$, of -1.1×10^{-2} deg/°C, as explained in the Experimental Section. The solid lines represent the linear least mean squares relationships for the data from the salt series, whereas the dashed lines represent the behavior of the data from the premelting series.

As observed in Figure 1, the negative band changes very little with temperature or salt content and the salt series data for this band have simply been averaged. These averages, as well as the slopes and intercepts for the straight lines defining the relationships for the positive band and the premelting data of the negative band, are given in Table II. The correlation coefficients for linear behavior are not especially impressive, but this can be attributed to the scatter of the CD data which is difficult to control. The slope for the premelting data from the positive band at the maximum is about 20% lower than that for the salt data. Although this discrepancy could, in principle, be attributed to an incorrect choice of $d\psi/dT$, this cannot be the entire answer since the differences between the premelting and the salt data are not consistent across the band. The discrepancies become greater at wavelengths above 275 nm and, conversely, less at wavelengths below. As a result, the behavior of the integrated data, $\int_{260}^{300}([\theta]/\lambda)\,d\lambda$, is comparable to that at 275 nm.

Table II: Characteristics of the Linear Dependence of $[\theta]_\lambda$ and Rotational Strength on $\Delta\psi$

	salt series			premelting series		
λ (nm)	m^a	b^a	r^b	m^a	b^a	r^b
275	−4.71	8.25	0.92	−3.83	7.93	0.98
270	−4.53	7.66	0.89	−4.67	7.10	0.97
265	−3.60	5.81	0.84	−3.90	5.31	0.91
260	−2.20	2.72	0.61	−2.36	2.11	0.63
280	−4.23	7.95	0.90	−3.31	7.61	0.98
285	−3.28	6.62	0.88	−1.23	6.61	0.53
290	−2.27	3.87	0.84	−0.75	3.97	0.33
245	0	−8.95		−0.428	−8.99	0.413
$\int_{260}^{300}\frac{[\theta]_\lambda}{\lambda}d\lambda$	−0.465	0.795	0.886	−0.372	0.758	0.986
$\int_{230}^{260}\frac{[\theta]_\lambda}{\lambda}d\lambda$	0	−0.680	0	−0.115	−0.683	0.852

[a] These symbols are the best fitting slopes (m) and intercepts (b) defined by the relationships $[\theta]_\lambda$ ($\times 10^{-3}$ deg cm²/dmol) or $\int([\theta]/\lambda)\,d\lambda = m\Delta\psi + b$. [b] The correlation coefficient r is from the linear regression analysis.

In view of the linear dependence of $[\theta]_{275}$ and $\int_{260}^{300}([\theta]/\lambda)\,d\lambda$ on $\Delta\psi$ in this limited range, we have extrapolated the value of $[\theta]_\lambda$ vs. $\Delta\psi$ at wavelength intervals of 2.5 nm for the salt series and the premelting series to values appropriate for the "C" reference spectrum obtained previously (Hanlon et al., 1975). The angles corresponding to values of $[\theta]_{275}$ [-0.5×10^3 deg cm²/dmol] and $\int_{260}^{300}([\theta]/\lambda)\,d\lambda$ (-0.081×10^3 deg cm²/dmol) for the C conformation were first determined using the data shown in Figure 2A,C. This value of $\Delta\psi$ was 1.87° for the salt series and 2.24° for the premelting series. The

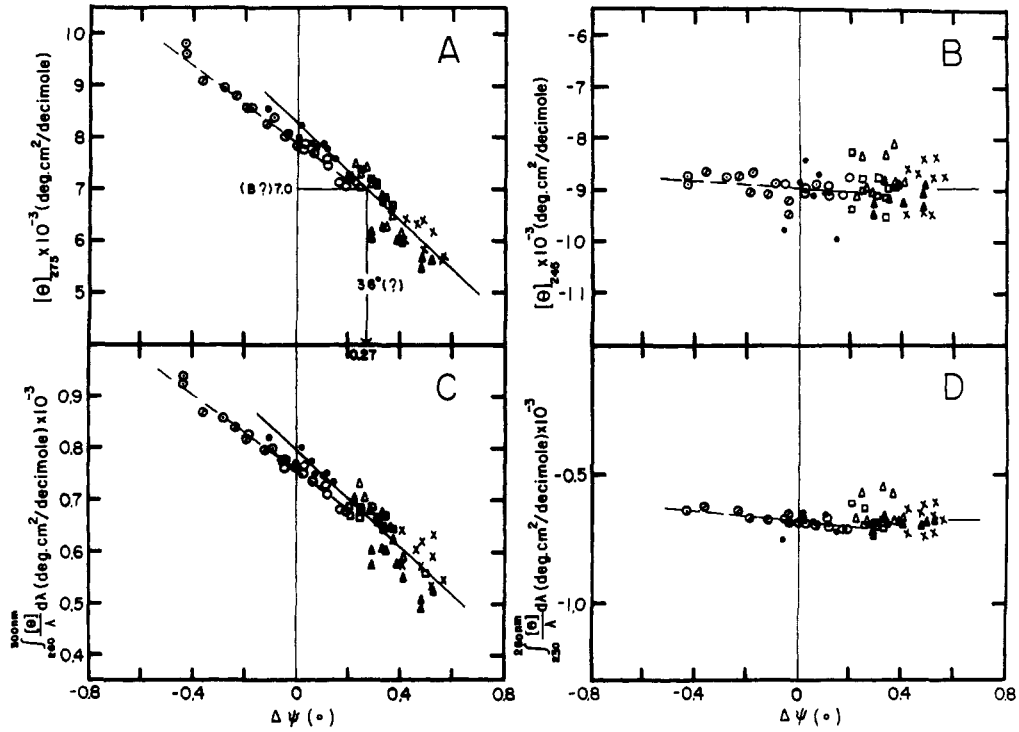

FIGURE 2: Dependence of the circular dichroism spectral properties of calf thymus DNA on the duplex winding angle, ψ. The linear relationships between values of $[\theta]$ and $\Delta\psi$ are presented in this figure for various salt solutions (salt series) and for various temperatures in NaCl solutions (premelting series). The data are coded identically in all panels as follows. Premelting series: 0.10 M NaCl (⊙), 0.02 M NaCl (⊘), and 0.05 M NaCl (○). Salt series: NaCl (●), KCl (□), LiCl (△), CsCl (▲), and NH_4Cl (×). The solid lines represent the linear least mean squares lines through all the salt data (Cary 60 and J-40A). The dashed lines represent the linear least mean squares lines through the premelting data (taken with the J-40A only). Panels A and B portray the behavior of single wavelength values of $[\theta]$ near the maximum of the positive band (275 nm) and the minimum of the negative band (245 nm), respectively. Panels C and D show the behavior of the function, $\int([\theta]/\lambda)\, d\lambda$, which is proportional to the rotational strength of the positive band (260–300 nm) and the negative band (230–260 nm). The value of $\Delta\psi = 0$ corresponds to the characteristics of DNA in 0.05 M NaCl at 21.5 °C. The position of $\psi = 36°$ has been tentatively identified in panel A as corresponding to a value of $\Delta\psi$ of 0.27°, as described under Results and Discussion.

remaining portions of the spectra were then extrapolated to these two values for the individual series using the linear least mean squares relationships of the dependence of each wavelength value of $[\theta]_\lambda$ on $\Delta\psi$. The results of these extrapolations are shown in Figure 3A,B as the solid beaded lines. The spectrum obtained from the salt data is quite close to the C reference spectrum (indicated by ×). A similar curve can be generated as a linear combination of any two members of the series, extrapolated to a value of $[\theta]_{275} = -0.5 \times 10^3$. The small difference (shown as the open triangles) between it and the C spectrum could easily be due to a small amount of A component (4 to 6%), whose transformation was linearly linked to B → C transition (and thus undetectable by our component analysis), and/or a refractive index difference between the aqueous solutions employed in these current experiments and the highly concentrated salt solutions employed in the previous studies.

As one might anticipate, in view of the results of the component analyses, the spectrum obtained from the premelting data is different from that obtained from the extrapolation of the salt data. If a C component is present, the difference between this spectrum and the normal C reference spectrum will reflect the shape of the additional spectral component. This difference, again shown as the open triangle curve, could arise from a red shift as the temperature decreases, but this clearly does not account for the entire difference since a simple red shift centered about 275 nm and contained entirely in the positive band would not have surfaced as an independent spectral component.

In view of the disparate behavior of the two halves of the positive band in the premelting series compared to the salt

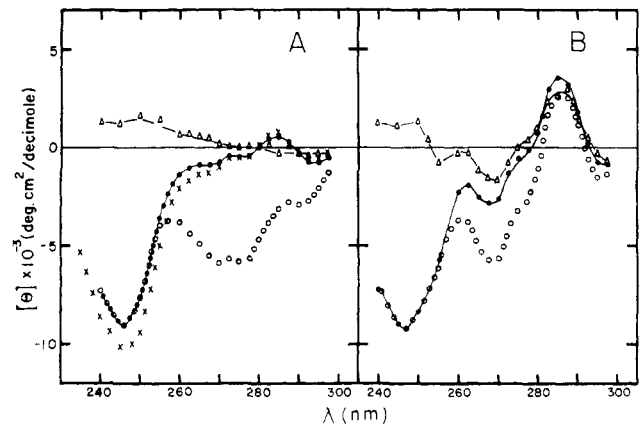

FIGURE 3: Extrapolated circular dichroism spectra of calf thymus DNA, based on the linear relationships between $[\theta]_\lambda$ and $\Delta\psi$. (A) The CD spectra for the salt series data at $\Delta\psi = 1.9°$ (●) and 3.0° (○). For comparison, the "C" reference spectrum (×), taken from Hanlon et al. (1975), is included. The difference between the $\Delta\psi = 1.9°$ and the C spectrum ($[\theta]_\lambda^{\Delta\psi = 1.9°} - [\theta]_\lambda^{C}$) is plotted (-Δ-). (B) The CD spectra for the premelting series data at $\Delta\psi = 2.2°$ (-●-) and 3.0° (○). The difference ($[\theta]_\lambda^{\Delta\psi = 2.2°} - [\theta]_\lambda^{C}$) is plotted (-Δ-).

experiments, it seems likely that, in addition to an overall conformational change, possibly involving movement along a B → C conformational continuum, there is superimposed an additional effect attributable to a change in the weighting factors associated with the GC and the AT contributions to the spectrum. In an analysis of the dependence of the CD spectrum of DNA on base composition, Gratzer et al. (1970) have obtained two reference curves, one for a hypothetical

100% GC component and the other for a 100% AT component. When mixed together in a linear manner, these two curves simulate reasonably well an actual CD spectrum of a DNA of a given GC content and random sequence. The major portion of the rotational strength of the positive band of the 100% GC component is contributed by the signals below 275 nm, while the reverse is true for the 100% AT reference. Since the premelting data exhibit less discrepancies between the slopes of the $[\theta]$ vs. ψ relationships below 275 nm, compared to the salt data, and greater discrepancies above 275 nm, we suggest that as the temperature increases the GC-rich regions of the spectrum continue to respond to the same extent as they had under the influence of ions. The response of the AT-rich regions, however, begins to lag behind, possibly because of increasing instability. These AT-rich regions are correspondingly unable to exert much of an effect on the overall conformation of the DNA molecule as the temperature increases, and hence the overall conformation begins to take on the character of a GC-rich DNA driven into a form whose helical parameters may exceed the classical B form (see discussion below).

One of the major difficulties with these experiments is the absence of absolute values of ψ, since all of the data are relative. If one calls upon data available in the literature, however, a reasonable guess as the position of $\psi = 36°$ can be made. On the basis of the results of wide-angle X-ray scattering experiments, Bram (1971) has concluded that calf thymus DNA in 1 M NaCl has a B conformation, with a ψ of 36°. At this concentration of NaCl, our previous experiments (Hanlon et al., 1975) reveal that $[\theta]_{275}$ for DNA is 7.0 $\times 10^3$ deg cm^2/dmol, which agrees very well with the value of $[\theta]_{275}$ of the spectrum of an unoriented film of calf thymus DNA at 92% relative humidity, identified as the spectrum appropriate for the B conformation by Tunis-Schneider & Maestre (1970). This value of 7.0 $\times 10^3$ deg cm^2/dmol corresponds to a $\Delta\psi = 0.27°$, and hence we have tentatively identified that position on the $\Delta\psi$ axis as 36°.

Using this as our reference position, the value of $\Delta\psi°$ corresponding to the C form of DNA (for which $\psi = 360/9.3 = 38.7°$) can be identified as $\Delta\psi \simeq 3°$. If the spectral data are extrapolated to this value of $\Delta\psi$, the spectra represented by the open circles in Figure 3A,B are obtained. As might be anticipated, the spectrum obtained from the premelting data is clearly different from that obtained from the salt data and has not been previously observed in other experiments.

In contrast, the spectrum at $\Delta\psi = 3°$ obtained from the salt series resembles in shape (although not in magnitude) a type of spectrum, the ψ spectrum, observed in other experiments involving DNA (Studdert et al., 1972; Jordan et al., 1972; Ivanov et al., 1973; Wolf et al., 1977). It is distinctly different, however, from that which we have identified as an end point in the B → C transition (Hanlon et al., 1975). One can offer several possible explanations for this. First, there is always the possibility that the spectrum we have identified as the C form in solution does not correspond to 100% C. We think this an unlikely explanation, however, since our "C" spectrum is similar to the spectrum of a film of DNA obtained as a conformational end point by Tunis-Schneider & Maestre (1970) under conditions where the C form exists in fibers, as determined by X-ray diffraction experiments (Marvin et al., 1961), and in film, as ascertained by linear IR dichroism (Brahms et al., 1973).

A second possibility for the discrepancy concerns the hazardous character of the extrapolation: the range of $\Delta\psi$ values over which the data were obtained is considerably removed from 3°, and the scatter in the data points is considerable. Furthermore, it might be argued that we have no assurance that the dependence of $[\theta]_\lambda$ on ψ remains linear over the 3° range. The B → C conformational transition involves more angular changes and helical parameters than just the rotational angle between the bases. These additional parameters could conceivably not be linked in the same manner as the conformational transformation proceeds. The evidence against this possibility, however, is the fact that the spectrum extrapolated as a linear function of ψ at $\Delta\psi = 1.87°$ is very close to that obtained in the studies previously cited (Tunis-Schneider & Maestre, 1970; Hanlon et al., 1975). If contributions of various helical parameters, such as tilt, twist, residues per turn, and distance of the bases from the helical axis, are uncoupled and thus make variable contributions at different values of ψ, an extrapolation of values of $[\theta]_\lambda$ from one unique range of ψ values should not yield the spectrum previously obtained.

We think that the most likely explanation for the failure to find a "C" spectrum at a value of ψ compatible with the C conformation is due to a constant factor error in $\Delta\psi$. The values of $\Delta\psi$ used in these experiments with calf thymus DNA were taken, as previously explained, from data on circular PM-2 DNA. Although the average GC content of the latter is the same as calf thymus, the repetitive sequences are apt to be different. More importantly, the circularity may impose certain constraints on the intact PM-2 molecule which will be absent in the linear heterogeneous calf thymus DNA. These constraints would cause values of $\Delta\psi$ based on the PM-2 data to be too small. As long as the superhelical densities are reasonably low, the error in $\Delta\psi$ is likely to be a linear function of the superhelical density of the PM-2 molecule. Hence, one would expect that $\Delta\psi_{\text{calf thymus}} = k\Delta\psi_{\text{PM-2}}$, where k is a constant of proportionality, appropriate for the range of $\Delta\psi$ used.

On the basis of the literature data, it is difficult to judge whether PM-2 DNA at the superhelical densities examined is small enough for the above to be a realistic possibility. Certainly, dramatic differences in the response of the CD spectra to changes in salt concentration are observed for lower molecular weight species such as ϕX174 DNA (3.4 $\times 10^6$) (Campbell & Lochhead, 1971) and *Escherichia coli* 15 plasmid (1.45 $\times 10^6$) (Maestre & Wang, 1971). The change in the value of $[\theta]_{275}$ in going from low salt to high salt concentrations for the intact circular form is about half the value observed for the nicked form. If $d[\theta]/d\psi$ is the same for both forms, as we have implicitly assumed in this work, then the value of $\Delta\psi$ for a given change in salt concentration is twice as large in the nicked form for these lower molecular weight DNA species. Higher molecular weight DNAs, however, such as λb2b5c DNA (26 $\times 10^6$), show little or no difference between the nicked and the intact state in their responsiveness to changes in the ionic environment (Maestre & Wang, 1971). Whether the intermediate molecular weight of PM-2 DNA (6.5 $\times 10^6$) is sufficient to impose a lower dependence of $\Delta\psi$ on ionic strength compared to a linear counterpart such as calf thymus can only be ascertained by direct experimental measurements. We are currently undertaking such experiments.

Conclusion

The spectral transformations in DNA induced by increasing salt content of the aqueous medium or decreasing temperature are clearly similar but not identical. There is a wealth of data to support the contention that the salt-induced transition reflects a conformational transformation from a B-like to a

more C-like state (Tunis-Schneider & Maestre, 1970; Hanlon et al., 1975). Other studies have suggested that the thermal effects on the spectrum of poly[d(AT)] can also be interpreted in terms of a similar transformation (Brahms et al., 1976), and, indeed, this was suggested earlier by Gennis & Cantor (1972) in their studies on the thermally induced changes in the spectra of DNA of different base compositions. The direction of the changes in ψ in each type of experiment (the salt series and the premelting series) supports these conclusions that a B → C type transformation is involved. The correlation between the values of $[\theta]_\lambda$ and ψ also confirms our earlier conclusions that the lowering of the positive band in the CD spectrum of DNA above 260 nm is associated with an increased C character.

Our data suggest, however, that although these two environmental factors, salt content and temperature, can act together to drive the DNA along a conformational continuum in which the B and C forms represent two points, temperature has a discriminatory effect on the relative contributions of the GC and the AT base pairs to the spectra, which is not found in the salt series. It is also clear from these and other experiments that the B form is not a conformational end point; the helical duplex can become underwound by perhaps as much as 1° beyond the B form, as a result of decreases in ionic strength and/or increases in temperature. The same may also be true for the C form, but we have no way of verifying this at the present time.

The similar role played by these two environmental factors can be rationalized using the same explanation which we have recently offered to account for the specific ion effect on the B → C conversion (Hanlon et al., 1978). In this latter study of the efficiency of a variety of divalent ions at dilute concentrations in effecting the structural conversion to the C form, we have suggested that the conformation of a given DNA molecule, in the absence of topological restraints, is primarily dictated by electrostatic factors. The state of hydration of the DNA molecule [which was demonstrated to be an important factor by our earlier study (Wolf & Hanlon, 1975)] and counterions both function as modulators of the electrostatic free energy which is actually the primary driving force for the conformational changes. When the electrostatic free energy of the base-stacked duplex is high, the most stable conformation is one which tends to minimize not only the major electrostatic repulsive interactions of the phosphate groups but also the repulsive interactions between the phosphates and the partial negative charges on the purine and pyrimidine bases. The preferred conformation under these conditions should be one in which the interstrand distance is high and the grooves of the helix are more or less equal in size, with the bases driven in toward the center of the helix. On a conformational continuum, this structure would be closer in characteristics to the B form than any of the other crystallographic forms. Increases in the electrostatic free energy will drive the molecule further along this continuum. Interstrand spacing is further increased until that point is reached where the magnitude of the repulsive interactions no longer permits the maintenance of the base-stacked helical duplex.

The electrostatic free energy of the DNA molecule is a complex function of cation association, ionic strength of the medium, and hydration of both the DNA molecule, the cations, and the DNA–cation complex. Lowering the ionic strength and, correspondingly, the cation concentration at a fixed temperature will increase the electrostatic free energy of the DNA molecule. An increase in temperature, at a fixed ionic strength and cation concentration, will also increase the electrostatic free energy of DNA as a natural consequence of increased counterion dissociation, reduction in hydration, and decreases in the dielectric constant of the aqueous solvent. Both these variables (salt content and temperature) should thus drive the molecule in the same general conformational direction, as has been observed to be the case.

Conversely, a reduction of the electrostatic free energy because of increases in cation concentration, ionic strength, or decreases in temperature (which promote site binding and increases in the dielectric constant) permits the closer approach of base sites and the ribophosphate backbone and thus favors the existence of other secondary structures whose characteristics include grooves of unequal size which are shallower (because the base pairs are off-axis) or narrower than the B-like form described above. Such forms would tend to be promoted by cations bound to DNA sites in such a way as to effectively screen the phosphate/phosphate and the phosphate/base repulsive interactions. At a given level of hydration, the efficiency of a given cation in inducing the structural transformation will be dictated by how well it fits into the heterogeneous set of sites consisting of the DNA bases and phosphate groups covered with a primary hydration shell. This latter, however, actually prevents maximally effective neutralization, and, hence, removal of this layer facilitates better cation association and more effective conversion to the alternate conformations. Thus, one finds a loss of the specific ion effect at a point where the DNA molecule begins to suffer a significant loss of its primary hydration shell, and further transformation from a B-like to the C form becomes dependent only on the hydration variable (Wolf & Hanlon, 1975).

A decrease in temperature at any point in this continuum tends to do something more than simply increase the C content at the expense of the B-like state. Our data suggest that there is also an increase in the AT contributions to the spectrum. If this is actually a conformational contribution (as opposed to a simple electronic contribution), then cations which have a preference for binding at AT sites will be more effective in inducing the conversion to the C form, since AT-rich regions are more readily transformed to the C conformation under given conditions of ionic strength and hydration (Pilet & Brahms, 1973). This would explain the greater efficiency of the NH_4^+ ion, which should have a greater preference for AT-rich regions, in effecting the B → C conversion (Hanlon et al., 1975; Wolf & Hanlon, 1975). Furthermore, if these conformational effects initiated at AT-rich sites are propagated over a considerable number of base pairs, then a number of intriguing allosteric mechanisms for the control of DNA interactions become possible.

In summary, the data from these experiments support the conclusion that both temperature and cations exert real conformational effects on DNA in solution. The character of these effects is consistent with the movement of the molecule along a conformational continuum in which the B and the C forms constitute two points, but apparently do not represent final end points. This conformational movement is correlated with changes in the positive band of the DNA spectrum above 260 nm. The specific ion effect observed in earlier studies (Hanlon et al., 1975) is thus seen to be a real effect on the conformation and not a simple perturbation of the electronic properties of the base chromophores.

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Influence of Different Levels of 2-Thiocytidine on Physical and Template Properties of Cytidine–2-Thiocytidine Copolymers†

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ABSTRACT: Copolymers containing cytidine (C) and various amounts of 2-thiocytidine (s^2C) were prepared using polynucleotide phosphorylase from *Micrococcus luteus*. Both CTP and s^2CTP polymerized equally well. The copolymers were used as templates for DNA-dependent RNA polymerase from *Escherichia coli* in the presence of Mn^{2+}, and it was found that, in contrast to the homopolymer poly(s^2C), the (C,s^2C) copolymers were active as templates in this transcriptional assay. When the amount of s^2C did not exceed approximately 10%, no difference in GMP incorporation compared to poly(C) was noted under the standard conditions of 2-h incubation. When the amount of s^2C was greater than approximately 10%, the decrease in template activity was proportional to the increase in amount of s^2CMP. The changes in template activity were paralleled by the change in the melting temperature of the "self-complementary" double strand. With increasing time of transcription the depressing effect of increasing amounts of s^2C in copolymers was diminished, and after 8-h incubation, transcription was similar to that of poly(C). When the template copolymer contained [^{14}C]C the [^{14}C]C–[^{3}H]G ratio of the double-stranded polymer changed with the amount of s^2C present, as expected, thus establishing the fact that base pairing between s^2C and G occurred. It is assumed that the presence of sulfur instead of oxygen causes the observed slower rate in enzymatic polymerizations with both polynucleotide phosphorylase and RNA polymerase but that this is not a critical factor for base recognition. A model is given to explain the stereochemistry of s^2C–G base pairing resulting in the formation of only two hydrogen bonds.

Our continuing study of the effects of alkylation on coding properties of pyrimidines in polynucleotides (Singer & Fraenkel-Conrat, 1970; Singer et al., 1978) is based on the assumption that there are very few modified bases in an in vivo modified nucleic acid. Enzymes involved in transcription would therefore generally recognize such nucleic acids as being structurally unchanged, and normal transcription would proceed until a modified base is reached. The decision as to whether or not a nucleoside becomes incorporated, and if so, which nucleoside, should depend only on the properties of the individual modified base. This is in contrast to the parameters for base pairing in modified homopolymers where the effect of secondary structure contributed by the modified base can

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