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Correlations between Deoxyribonucleic Acid Structural Parameters and Calculated Circular Dichroism Spectra[†]

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ABSTRACT: Circular dichroism spectra are calculated for 68 different conformations of the bases in the double-stranded sequence of T-G-G-C-G-T-A-T-T-C-T and its complementary strand. All conformations are right-handed helices and include both published models and energy-minimized forms. Examination of the dependence of the circular dichroism band at 275 nm shows that the magnitude of the band is a linear function of both the helix winding angle and the base pair twist. Correlations of the magnitude of the circular dichroism with the distance between base pairs, the distance of the base pairs from the helix axis, or the tilt of the base pair relative to the helix axis are poorer.

When Watson and Crick first proposed that deoxyribonucleic acid (DNA) existed in a right-handed double helix, two different forms of the molecule, corresponding to different salt and humidity conditions, had already been discovered by using X-ray diffraction on fibers. These two forms, later called the B form (Langridge et al., 1960) and the A form (Fuller et al., 1965), have since been joined by other forms derived from fiber diffraction studies: the B′ form (Arnott & Selsing, 1974), the C form (Marvin et al., 1961), the D form (Davies & Baldwin, 1963), and the T form (Mokulsky et al., 1972). Similar studies carried out on double-stranded ribonucleic acid (RNA) have demonstrated that this class of nucleic acids exists in two distinct forms, both similar to A form DNA: A RNA (Arnott et al., 1967) and A′ RNA (O'Brien & MacEwan, 1970). All these forms were helices with the right-handed screw sense. Recently, a left-handed structure, obtained from X-ray diffraction studies of a crystal of the self-complementary fragment d(C-G-C-G-C-G), has been proposed (Wang et al., 1979). Diffraction studies on poly[d(GC)]·poly[d(GC)] have also suggested the occurrence of this left-handed structure, Z DNA, in fibers (Arnott et al., 1980).

By use of the limited information obtained from fiber diffraction work, together with stereochemical information available from crystal studies of the nucleosides and nucleotides, rather detailed models for these forms have been constructed and refined. While all the models except Z DNA are similar in that they are right-handed helices and the strands are antiparallel, many differences exist among them. One of the major differences dividing the B form from the A form involves the sugar pucker in the helix backbone (Arnott & Hukins, 1972; Ivanov et al., 1973). In the B form it is 3′-exo (Arnott & Hukins, 1972); other forms with this pucker, or the very similar 2′-endo, comprise the $\bar{\text{B}}$ family of interrelated forms. These include B′, C, D, and T DNA (Ivanov et al., 1973). The sugar pucker in the A form and its relatives is 3′-endo (Arnott & Hukins, 1972). Other members of this $\bar{\text{A}}$ family are A RNA and A′ RNA (Ivanov et al., 1974). Another major difference among the different forms is found in the helix winding angle, τ, which, for DNA, ranges from 32.7 to 45° (Table I).

In addition to the winding angle, four other parameters are required to uniquely describe the relative position of the bases within the helical array (Arnott, 1970). As shown in Figure 1, these additional parameters are the shift of the base pair along the helix axis (h), the displacement of the bases from the helix axis (D), the tilt of the base pair relative to the helix axis (TL), and the relative twist of the base planes in the base pair (TW).

While all the models above were derived from diffraction studies, the majority of nucleic acid studies have been, and continue to be, carried out in solution. Therefore, it was reasonable to inquire as to which of the above forms (if any) were assumed by nucleic acids in solution. The optical technique most sensitive to changes in nucleic acids' conformations is circular dichroism (CD). The crucial linkage between the structural models gleaned from X-ray studies and changes in the CD spectrum was provided by Tunis-Schneider & Maestre (1970), who obtained CD spectra for calf thymus DNA films prepared under conditions of humidity and ionic strength similar to those employed in the fiber studies. They determined that the CD spectrum in the range from 240 to 300 nm progressed from a positive nonconservative spectrum, through a conservative shape, to a final negative nonconservative shape as the conditions varied from those favorable for the A, B, and C forms of DNA, respectively. With this information, it was possible to relate changes in the CD spectrum to qualitative changes in the DNA conformation, but the more difficult question of any explicit dependence of the spectrum upon one or more of the five helical parameters remained unanswered.

The first attempts to discover these explicit dependences were carried out by calculations of nucleic acids' CD spectra for different conformations of the molecules. Johnson & Tinoco (1969) derived a simple method for calculating the CD of a polynucleotide by using exciton theory. Using X-ray data available at the time, they were able to re-create the CD curves for the A, B, and C forms of DNA (although the agreement with experiment was not as good in the case of the C form as it was for the other two). They noted that the chief factor in the difference between the A form and B form CD spectra apparently arose from the larger tilt of the base pair plane in the former. Later, Moore & Wagner (1973) applied the method to helix geometries derived from the A RNA, B DNA, and C DNA forms by removing any base pair tilt and twist, by varying the base pair displacement at zero tilt and twist

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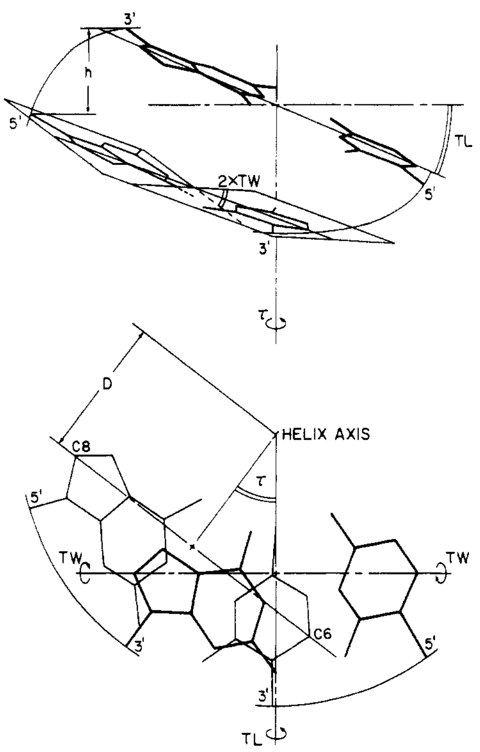

FIGURE 1: Five structural parameters necessary to specify relative base positions in the nucleic acid double helix: winding angle, τ; shift of bases along helix, h; displacement of base pairs from helix axis, D; tilt of a base pair relative to helix axis, TL; twist of the two bases in a base pair, TW. In this figure, the helix is right-handed, the displacement and twist are positive, and the tilt is negative; the conventions for the signs of twist and tilt are opposite to those in Arnott, 1970.

angles, and by changing the number of base pairs per helical turn (τ) at a fixed displacement. They concluded that the difference in the spectra for these forms arose largely as a result of the different displacement distance from the helix axis for each form.

An early experimental study of the effect of salt and solvent on the CD of calf thymus DNA by Ivanov et al. (1973) first established a possible correlation between the magnitude of the CD at 280 nm and the winding angle. Baase & Johnson (1979), using circular DNA, and Chan et al. (1979), using linear DNA at different salt concentrations, found quantitative correlations between the CD magnitude at 275 nm and τ. In both cases, the nucleic acid G plus C contents were equal to 42% (Chan et al., 1979), and conditions for the experiments placed the winding angles for each in the range of values associated with the B and C forms, although an exact assignment of a particular value for τ could not be made.

We wish to report a new study of the relations between nucleic acid conformations and the circular dichroism. In this study, a series of conformations in the right-handed helical sense, derived from both the X-ray fiber diffraction models and energy-minimization studies (Zhurkin et al., 1978), is used to calculate the CD of an 11 base pair sequence of DNA by a matrix method which includes all interactions between electric transition moments of the bases (Bayley et al., 1969; Bayley, 1973).

Methods

All calculations of the circular dichroism were carried out for the 22-base double-stranded sequence of

5′ T-G-G-C-G-T-A-T-T-C-T 3′
3′ A-C-C-G-C-A-T-A-A-G-A 5′

This particular sequence was chosen because it contains all 10 possible nearest-neighbor combinations, considered two base pairs at a time, for double-stranded nucleic acids.

Since the circular dichroism of the nucleic acids is largely a function of the helical arrangement of the base pairs (Moore & Wagner, 1974; Sprecher et al., 1979), only the bases were considered. Base coordinates for the diffraction-derived forms were taken from the literature (see Table I). Other forms used in the calculations for the $\bar{A}$ and $\bar{B}$ families were the energetically favorable forms found by Zhurkin et al. (1978), together with forms obtained from these by slight variations of the helix parameters. The limits of the variations correspond to their thermal fluctuations (Zhurkin et al., 1980): $|\Delta TL|$ (tilt) = 5–10° and $|\Delta TW|$ (twist) = 5–10°. No variations were introduced in the other three parameters, so up to 10 slightly different conformations were obtained for any one set of τ, h, and D values. In all, CD calculations were carried out on 68 different conformations typical of either the $\bar{A}$ (18 conformations) or $\bar{B}$ (50 conformations) families.

The calculation of the CD for all conformations was carried out by using standard methods (Bayley et al., 1969; Bayley, 1973). Monomer electronic properties employed were those selected by Cech for absorption bands above 200 nm (Cech et al., 1976; Cech & Tinoco, 1977). A background of three mutually perpendicular polarizabilities was used to account for transitions below 200 nm (Cech et al., 1976); with the exception of one of these background oscillators, no out of plane transitions were considered. Monomer magnetic dipoles also were not included.

The results of the matrix calculations were lists of transition energies and rotational strengths. In order to plot a spectrum of $\Delta\epsilon$ ($=\epsilon_L - \epsilon_R$) vs. wavelength over the range of 200–350 nm, we gave each transition a Gaussian shape having a line width of 1500 cm^{-1}. Selected wavelengths were then chosen for further study of the CD trends with changes in the helix parameters. Since the large numbers of similar conformations in the minimized energy subgroups usually produced very similar values for $\Delta\epsilon$, all 68 calculated points were not plotted for the $\Delta\epsilon$ vs. τ, h, and D cases. For each subgroup with a particular τ, h, and D, data were plotted only for the two conformations utilizing the median value for tilt coupled with the extremes for twist and the two in which the median value for twist is coupled with the extremes for tilt. When such a selection was made, the effective range of calculated $\Delta\epsilon$ values was considered and each subgroup of τ, h, and D was weighted equally.

Results

All 68 plots of the calculated CD spectra contained similar features in the 240–320-nm region: a positive CD band at longer wavelengths crossing over between 250 and 280 nm to a negative CD band above 240 nm. The positive CD band decreased in magnitude, while the negative band grew in magnitude in a regular fashion as the helix conformation progressed through the sequence of A, B, C, and D forms. Below 240 nm there appeared in each case a relative maximum and relative minimum whose signs varied with conformation in no discernable order. Plots of CD magnitude at 300, 275, 250, 240, 237.5, and 225 nm vs. each of the five helix parameters were made. The magnitudes of the CD at 275 nm were judged to be the most credible, since the electronic properties of the bases are better known in this region of the UV. In the discussions which follow, variations of the CD solely at this wavelength are considered.

The experimental and calculated values for the CD at 275 nm are presented in Table I for the A, B, B′, and C forms of

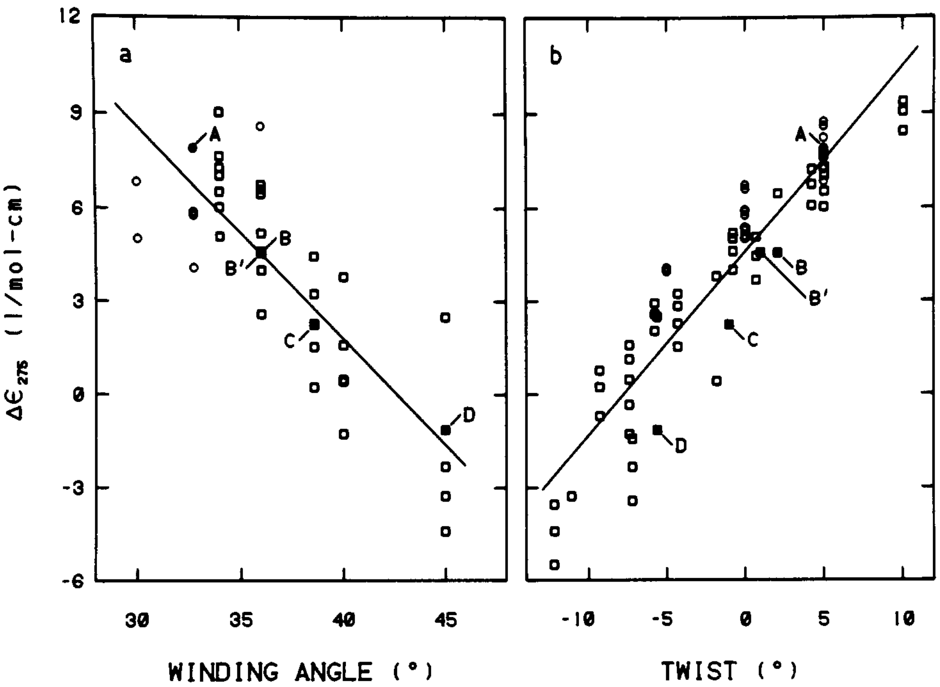

FIGURE 2: Variation of calculated CD magnitudes at 275 nm ($\Delta\epsilon_{275}$) with (a) winding angle, τ, and (b) twist, TW. Data from the $\bar{A}$ family are denoted by circles and data from the $\bar{B}$ family, by squares. Filled and labeled points are derived from the published models of DNA. Lines represent the best least-squares fit to data.

Table I: Helix Parameters[a] and CD Magnitudes for Known Forms of DNA

form	τ (deg)	h (Å)	D (Å)	tilt (deg)	twist (deg)	$\Delta\epsilon_{275}$[L/(mol cm)] exptl[b]	this work
A	32.7	2.56	4.5	−19.5	5.9	10.5	7.9
B	36	3.38	−0.2	5.9	2.1	2.3	4.6
B′	36	3.29	−0.1	7.9	1.0	2.7	4.6
C	38.6	3.31	−0.9	8.0	−1.0	−0.7	2.3
D	45	3.03	−1.8	16.0	−5.6		−1.1

[a] A form (Arnott & Hukins, 1972); B, B′, C, and D forms (Arnott & Selsing, 1975). [b] B′ form (Wells et al., 1970); A, B, and C forms (Sprecher et al., 1979).

DNA. As can be seen, the agreement between the calculations and experiments is only fair, particularly for the C form where the incorrect sign is predicted for $\Delta\epsilon$. However, the trend in values is correct; the A form has the largest calculated and observed CD, whereas the C form has the smallest calculated and observed CD. Possible reasons for this discrepancy between theory and experiment include the effect of base sequence, the accuracy of the monomer electronic properties employed in the calculations, and the fact that DNA in solution may not have the same structure that is does in fibers (Wang, 1979).

With the exception of the B′ form, which comes from poly(dA)·poly(dT) data (Wells et al., 1970), the experimental values are obtained with calf thymus DNA (Sprecher et al., 1979), which has a G plus C content similar to our sequence (42% vs. 45%). Cech & Tinoco (1977) noted that runs of three or more identical base pairs give rise to strong exciton-type coupling of identical transitions, altering the CD patterns from those which might occur if only two adjacent pairs were identical. Since our sequence contains runs of four G·C and four A·T base pairs, this exciton effect might be expected to contribute to the pattern. However, in further study of five "random" sequences of 10–20 base pairs, Cech (1975) found little difference in the calculated CD's. Calculations on permutations of the sequence used here showed little difference in the CD results for each geometry (A, B, and C forms). However, calculations on sequences of 21 base pairs showed that significant changes in CD magnitudes could occur as the sequence of the bases changed. Thus, while the sequence of bases used here may have introduced some bias in the calculated CD magnitudes, it is likely to be small within the limitations imposed by only considering the 10 possible nearest-neighbor contributions.

A more likely source of error, the choice of monomer electronic properties, has been discussed by Cech and coworkers (Cech et al., 1976; Cech & Tinoco, 1977). Improved agreement with experiment might be achieved by exploring alternate assignments of transition energies and transition directions. However, here we are more interested in examining the change in the circular dichroism with changes in the helix geometry, and we believe the underlying theory of the factors responsible for nucleic acids' CD (i.e., the geometrical dependence of the interactions between helically arranged bases) is sound, so that the theory is capable of predicting these changes. Thus, whereas the absolute magnitudes of the calculated CD at 275 nm may be incorrect, changes in magnitude with changes in the helix geometry are much more likely to be real.

Plots of the calculated magnitude of the CD at 275 nm ($\Delta\epsilon_{275}$) vs. each of the five helix parameters are shown in Figures 2, 3, and 4. Each plot identifies data derived from geometries of either the $\bar{A}$ or $\bar{B}$ families and also from the five published forms of Table I: A, B, B′, C, and D DNA. It is apparent that there is a definite correlation between $\Delta\epsilon_{275}$ and both τ and twist, extending over the range of both the $\bar{A}$ and $\bar{B}$ families. For $\Delta\epsilon_{275}$ vs. h and D, any correlation apparently occurs only within the $\bar{B}$ family of structures, while for $\Delta\epsilon_{275}$ vs. base pair tilt, no reasonable correlation appears. These observations are reflected in the least-squares fits to a line for each plot tabulated in Table II.

Any correlation between the magnitude of the circular dichroism at 275 nm and the parameters describing the double helix is a complicated one, as can be seen in Figures 2, 3, and

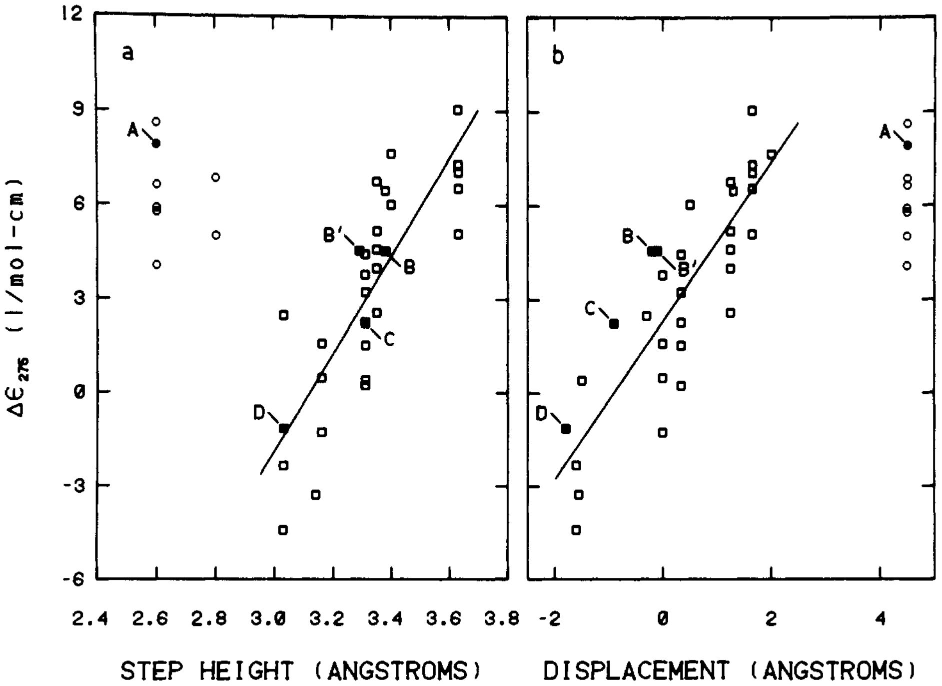

FIGURE 3: Variation of calculated CD magnitudes at 275 nm ($\Delta\epsilon_{275}$) with (a) height between bases, *h*, and (b) displacement, *D*. Data from the $\bar{A}$ family are denoted by circles and data from the $\bar{B}$ family, by squares. Filled and labeled points are derived from the published models of DNA. Lines represent the best least-squares fit to the $\bar{B}$ family data.

Table II: Least-Squares Fits to a Line of $\Delta\epsilon_{275}$ with Helix Parameters

parameter	slope[a]	intercept	no. of points	linear correlation coeff
τ	−0.68 ± 0.08	29 ± 3	39	−0.83
h	16 ± 2[b]	−49 ± 7[b]	31	0.82[b]
D	2.5 ± 0.3[b]	2.3 ± 0.3[b]	31	0.85[b]
tilt	−0.17 ± 0.03	3.2 ± 0.4	68	−0.56
twist	0.59 ± 0.03	4.6 ± 0.2	68	0.92

[a] Units are L/(mol cm deg) for τ, tilt, and twist and L/(mol cm Å) for *h* and *D*. [b] Data from $\bar{B}$ family only.

4. While it can be argued that these curves demonstrate some degree of correlation for all five parameters, we believe they can be classified in the three groups mentioned above: those with good linear correlation for both the $\bar{A}$ and $\bar{B}$ families ($\Delta\epsilon_{275}$ vs. τ and $\Delta\epsilon_{275}$ vs. twist), those with good linear correlation within the $\bar{B}$ family only ($\Delta\epsilon_{275}$ vs. *h* and $\Delta\epsilon_{275}$ vs. *D*), and those with poor linear correlation ($\Delta\epsilon_{275}$ vs. tilt).

For the plots of $\Delta\epsilon_{275}$ vs. τ and $\Delta\epsilon_{275}$ vs. twist (parts a and b, respectively, of Figure 2), a clear-cut correlation between the calculated CD and the helix parameters exists for all data points considered together. Linear least-squares fits show a high correlation considering the number of points involved (Table II). No clear break between points in the $\bar{A}$ and points in the $\bar{B}$ families occurs. Experimentally derived results for the correlation between the long wavelength band in the CD of DNA and the winding angle are readily compared with our results. Baase & Johnson (1979), using the circular DNA of PM2 phage, obtained a biphasic relation between $\Delta\epsilon_{275}$ and the winding angle in the neighborhood of the B form; we calculate a slope of −1.5 L/(mol cm deg) for the left-hand section of their curve and −9.0 L/(mol cm deg) for the right-hand section. Since our data extend over a much wider range of winding (15° vs. <1°), we think a more realistic comparison may be obtained by calculating a slope for all of Baase and Johnson's data; when this is done, a slope of −3.6 L/(mol cm deg) is obtained, vs. the value of −0.68 L/(mol cm deg) obtained here. Chan et al. (1979) also examined the relationship between $\Delta\epsilon_{275}$ and τ for linear calf thymus DNA and obtained slopes of −1.4 L/(mol cm deg) and −1.2 L/(mol cm deg) for different sample conditions. Again, their effective winding angle range is small (<2°).

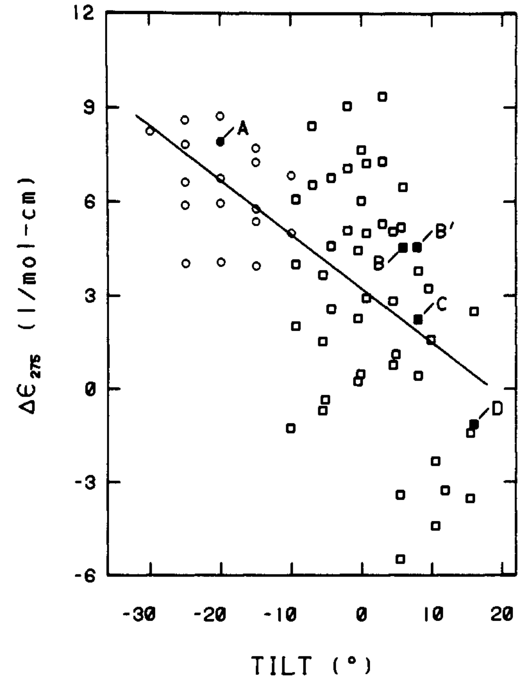

FIGURE 4: Variation of calculated CD magnitudes at 275 nm ($\Delta\epsilon_{275}$) with the tilt of the base pair, TL. Data from the $\bar{A}$ family are denoted by circles and data from the $\bar{B}$ family, by squares. Filled and labeled points are derived from the published models of DNA. The line represents the best least-squares fit to the data.

The outstanding feature of the plots for $\Delta\epsilon_{275}$ vs. *h* and $\Delta\epsilon_{275}$ vs. *D* (parts a and b, respectively, of Figures 3) is the division of the data groups between the $\bar{A}$ and $\bar{B}$ families. Here we obtained linear fits for the data derived from the $\bar{B}$ structures only; inclusion of the $\bar{A}$ family is clearly not warranted by virtue of the sharp break between the two data sets. For the case of dependence of the CD upon displacement of the bases from the helix axis, our results show qualitative agreement with those obtained by Moore & Wagner (1973), namely, that increasing the displacement increases the magnitude of the CD band at longer wavelength or, as they stated, the B form grows less conservative.

In the plot of $\Delta\epsilon_{275}$ vs. tilt (Figure 4), the difference between data from the $\bar{A}$ and $\bar{B}$ families also occurs but is obscured by the large scatter of the data. This can be seen by extrapolating the trend within the $\bar{B}$ family to tilt values observed for the $\bar{A}$ family; the calculated points for the $\bar{A}$ family fall below a trend line derived from $\bar{B}$ family points. Although any attempt to fit all data to a straight line shows a low linear correlation coefficient (see Table II), in a broad sense, we can state that as the base pair tilt passes from negative to positive values, the magnitude of the CD decreases. This is readily seen by only considering the points calculated for the known forms, which place the A form, having the more negative tilt, at the upper end of the calculated magnitudes, while the D form, with a large positive tilt, is near the lower end of the magnitude range.

Discussion

There are two problems which are interesting to consider in connection with the CD dependence on the bases' parameters. (1) How does each parameter control the CD magnitude irrespective of the energy of the helix and the values of the remaining parameters? This task was partially studied in the previous papers (Johnson & Tinoco, 1969; Moore & Wagner, 1973) and, to a limited extent, can be studied in this paper. (2) What is the dependence of the CD on the helix parameters as they *all* change to produce the different energetically reasonable double-stranded helices? This problem is addressed here for the first time.

In calculating the CD spectra for both the energetically minimized forms and their relatives obtained by allowing tilt and twist to vary within the limits of thermal fluctuations, we have obtained data which allow us to examine the dependence of the CD magnitude at 275 nm on either tilt or twist while holding the other four parameters constant. In a typical case, using the energy-minimized B form ($\tau = 36°$, $h = 3.35$ Å, $D = 1.25$ Å, TL = $-4.35°$, and TW = $-0.75°$) with ΔTL = $\pm5°$, $+10°$ and ΔTW = $\pm5°$, several features are evident. The trend in each case is the same: as the angle of tilt (twist) increases, the magnitude of the CD at 275 nm increases. The magnitude of the trend in each case is different, however. For twist the slope is $\sim$0.45 L/(mol cm deg), while for tilt it is only $\sim$0.1 L/(mol cm deg), indicating a much greater dependence of the magnitude upon twist rather than on tilt. The slope for tilt dependence disagrees with the conclusions reached by Johnson & Tinoco (1969), who found that increasing tilt angles lead to decreasing CD magnitudes in the long wavelength band. It is only when the magnitudes of the calculated CD's from many different conformations are plotted vs. tilt angle that the pattern seen by Johnson and Tinoco is established (see below). This discrepancy for the tilt dependence of the CD when all other parameters are held constant tends to underscore the difficulties involved in answering the first question posed above, even when dealing with parameters derived from energetically favorable conformations.

Most of our results are better suited to the discussion of the second question stated earlier: How does the CD vary as all the parameters vary within an energetically reasonable multidimensional space? It is to this question that the rest of this discussion is directed.

Our result establishing the strong dependence of the CD on the helix winding angle is consistent with the experimental data (Baase & Johnson, 1979; Chan et al., 1979). There is a strong correlation between the two factors, and the trends are the same: as τ increases, the magnitude of the long-wavelength band decreases. Although the slope we obtain is smaller than the experimental values by a factor of 2–5, we think this is due more to the wider range of winding angles we were able to examine in the calculations (15° vs. <2°) than to any significant error in either the experiments or CD theory; with further experimental work at different winding angles, closer agreement may be obtained.

In addition to a strong correlation between the CD and τ, we have also uncovered a strong correlation between the CD and the twist of the base pair. In a qualitative sense, this is in agreement with Moore & Wagner (1973), who showed that as twist was reduced to zero for a form, the magnitude of the CD at 275 nm decreased. However, since they reduced the base pair tilt and twist simultaneously, any change in the spectrum could not be attributed to one parameter alone. In light of our findings, we can say that the changes are more likely due to the reduction of the twist, rather than the tilt.

Since our results show strong correlation between $\Delta\epsilon_{275}$ and both the helix winding angle and base pair twist, it is reasonable to ask if correlation between the winding angle and twist occurs within the helix. In other words, is a change in the helix winding angle necessarily accompanied by a change in the twist of the base pairs, and do the changes maintain the same relationship throughout the possible ranges of values for each? Examination of these two parameters for the known forms in Table I suggests that, indeed, such a correlation does exist. As the winding angle progresses from 32.7 to 45°, the corresponding base pair twist angles change from 5.9 to $-5.6°$ in an ordered fashion. When the dependence of the winding angle and base pair twist is further examined by considering all possible data pairs employed in our calculations, the trend remains the same (increasing winding angles lead to decreasing twist angles), although the overall correlation is poor. This suggests that the two parameters are closely related: a change in one synchronously produces a change in the other.

Now that correlations between $\Delta\epsilon_{275}$ and both τ and twist have been shown, the reason for the break between the $\bar{A}$ and $\bar{B}$ families in the other parameter plots is easier to understand. As can be seen in Table I, when the winding angle increases, three of the remaining four helix parameters vary monotonically also: D, tilt, and twist. Only the height between base pairs does not follow this trend—there is a distinct break between the $\bar{A}$ and $\bar{B}$ family trends. Therefore, if any correlation exists for $\Delta\epsilon_{275}$ and h over the entire range of the possible values, none can exist for the other four parameters, or, as in our case, if one or more of the four "linked" parameters correlates with $\Delta\epsilon_{275}$, the height, h, cannot. This accounts for the sharp break between families seen in Figure 3a.

In previous study by Arnott et al. (1975), a relationship among the three helix parameters h, tilt, and twist was discovered in data from a dozen model structures. These workers found that the angle between the normal to a base plane and the helix axis (termed γ) was a linear function of the rise per residue (h). The angle γ is a function of both tilt and twist and is readily calculated by using eq 1. Applying (1) to the

$$\cos\gamma = \cos \mathrm{TL} \cos \mathrm{TW} \qquad (1)$$

conformations employed in creating Figure 3a (with the restriction that energy-minimized conformations derived from presumed thermal fluctuations in tilt and twist are ignored), we have found a roughly linear correlation between γ and h

also. We thus expect a plot of $\Delta\epsilon_{275}$ vs. γ to exhibit behavior similar to that seen with $\Delta\epsilon_{275}$ vs. h: separate and distinct groupings of points by family ($\bar{\text{A}}$ or $\bar{\text{B}}$) and a linear correlation for $\Delta\epsilon_{275}$ vs. γ with the $\bar{\text{B}}$ family points. It may be that the discontinuities between $\bar{\text{A}}$ and $\bar{\text{B}}$ families observed in the $\Delta\epsilon_{275}$ vs. tilt and $\Delta\epsilon_{275}$ vs. h plots are both related to their linkage through the parameter γ.

The reason for the discontinuity seen with displacement is less clear-cut, but it is encouraging to note that the direction of the trends established in the variation of CD magnitude with changes in this parameter (and also tilt) is qualitatively maintained as the $\bar{\text{B}}$ family values pass into those of the $\bar{\text{A}}$ family, only the magnitudes of the slopes vary. This is in keeping with the argument given above on the linkage seen between the four parameters in the known forms.

Previous calculations on the CD of DNA emphasized the effect of the tilt of the base pairs (Johnson & Tinoco, 1969) and the displacement of the base pairs from the helix axis (Moore & Wagner, 1973) on the conservative or nonconservative nature of the spectrum. We find that the trends in the CD implied by those studies are also followed in the present study. As the angle of tilt proceeds from positive to negative values, the magnitude of the long-wavelength band increases; as the displacement proceeds from negative to positive values, the magnitude of the long-wavelength band increases. However, the degree of correlation for each is much less than those we observe for the winding angle and base pair twist dependencies. In the earlier work, only one or two parameters were varied at one time, while the rest were constrained to remain constant; the conformations thus obtained could be energetically unfavorable, or impossible. In this study, all parameters were varied simultaneously so as to maintain energetically favorable conformations (Zhurkin et al., 1978). Use of these conformations in calculations is a more realistic method of examining any dependence of the CD upon helix parameters.

On the basis of this work, we can say with some certainty that measured changes in the 275-nm CD band of a DNA sample are linear functions of changes in the helix winding angle and the base pair twist: as the winding angle increases, the twist angle decreases, and the magnitude of the band decreases. While the variation of the CD with winding angle has been demonstrated experimentally, the interdependence of the winding angle and base pair twist meant that the twist was also changing. Calculational methods may well be the only practical means of investigating the geometrical dependence of double-stranded nucleic acids' circular dichroism.

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