Backbone Conformational Change in the A → B Transition of Deoxyribonucleic Acid†

Tom Kursar and G. Holzwarth*,‡

ABSTRACT: Infrared linear dichroism studies of A- and B-DNA films reveal six bands between 2800 and 3000 cm^{-1} which must arise from deoxyribose and thymine methyl CH stretching motions. The band at 2890 is perpendicularly polarized in A-DNA but parallel polarized in B-DNA. This band most probably originates in the $C'(5)H_2$ symmetric stretch; the polarization flip is consistent with the structural change occurring at C'(5) during the A → B transition, according to models derived from x-ray work.

The conformation of A- and B-DNA have been elucidated primarily through analysis of x-ray diffraction (see Arnott, 1970). In Figure 1 we show the deoxyribose-phosphate chain according to the most recent A and B models of Arnott. The backbone chain conformation is substantially different in the two structures. Because only fibers, rather than crystals, can be studied in x-ray work on DNA and few of the diffraction data correspond to periodicities less than 2.5 Å, the models proposed from the x-ray work for A- and B-DNA retain some uncertainties. For example, the conformation of the deoxyribose ring is difficult to establish (Arnott and Hukins, 1972a, 1973). In this situation infrared linear dichroism studies on DNA films can provide useful information on the orientation of particular groups in the polymer.

Previous work on the ir[1] linear dichroism (LD) of DNA and polynucleotide films (Fraser and Fraser, 1951; Sutherland and Tsuboi, 1957; Bradbury et al., 1961; Falk et al., 1963; Tsuboi, 1969; Pilet and Brahms, 1973; Champeil et al., 1973; Nishimura et al., 1974; Pilet et al., 1975) has focused primarily on the orientation of the bases and the phosphate group with respect to the helix axis, because the stretching modes of these groups exhibit strong dichroism. Reviews have been published of the infrared LD work performed prior to 1972 (Tsuboi, 1969; Tsuboi et al., 1973; Hartman et al., 1973). The most recent experimental work (Pilet and Brahms, 1972, 1973; Champeil et al., 1973; Nishimura et al., 1974) shows that the dichroism of the symmetric phosphate stretch at 1090 cm^{-1} changes from perpendicular to parallel in the B → A transition, in qualitative accord with predictions from B- and A-DNA models derived from x-ray work. The orientation of other chemical groups should be amenable to the same type of ir examination.

We have recently constructed an instrument capable of measuring infrared linear dichroism to much higher precision than was available to the earlier workers. In the present paper we report spectra obtained with this instrument for A- and B-DNA films. Our most notable findings are six previously unobserved bands, between 2800 and 3000 cm^{-1}, arising from ribose and thymine methyl C-H stretching modes. The data can test structural models for DNA.

Materials and Methods

Sample Preparation. Calf thymus DNA (Sigma) was dissolved to a concentration of 1.7 mg/ml in 40 mM phosphate buffer, pH 7, and 40 mM KF (μ = 0.10). After 48 h with stirring at 3 °C, undissolved polymer was removed by centrifugation. The solution was dialyzed to equilibrium against 5 mM NaCl (pH 7) and then lyophilized. Chloride was measured spectrophotometrically by the hexachloroferrate(III) method of Kupke and Sauer (1970).

Films were prepared from viscous solutions of DNA (20 mg per ml) to which had been added 3.5 mg of NaCl per g of DNA (Rupprecht and Forslind, 1970). A few drops of this solution were placed on CaF_2 or Irtran 2 discs and stroked unidirectionally until dry. The disc was placed in an airtight cell with the humidity fixed by an appropriate saturated salt solution (International Critical Tables).

The B to A conformational transition of DNA is obtained by changing the humidity from 92 to 75% relative humidity (Franklin and Gosling, 1953). We found that the transition will occur reproducibly if the sample film is warmed to 45 °C for 3 h in the presence of the vapors of the salt solution giving 75% relative humidity at 22 °C. This treatment introduces no denaturation as measured by the intensity of the 1710 cm^{-1} band. Our spectral observations of the B → A transition are in accord with work by Bradbury et al. (1961), by Pilet and Brahms (1973), and by Nishimura et al. (1974) in the 1090 and 1250 cm^{-1} regions. In the absence of warming we found that films often failed to exhibit a humidity-induced B → A transition (see Sutherland and Tsuboi, 1957; Falk et al., 1963).

Linear Dichroism. Infrared LD spectra were measured by a specially constructed polarization-modulation instrument. In this instrument a filter-grating monochromator is used to generate a beam of monochromatic infrared radiation. A wire-grad polarizer and germanium photoelastic modulator modulate the polarization of the monochromatic beam such that the radiation falling on the oriented sample alternates between horizontal and vertical polarization at 24 kHz. The beam illuminates approximately 0.5 cm^2 of sample. Sample temperature is 20–25 °C. If the sample is dichroic it will modulate the beam intensity at 24 kHz. After traversing the

† From the Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, Illinois 60637. *Received February 26, 1976.* This work was supported by National Science Foundation Grant GP8566 and by Grant NS07286 from the U.S. National Institutes of Neurological Diseases and Stroke. T.K. has received support from U.S. Public Health Service Training Grant GH780; G.H. was recipient of a Research Career Development Award from the NIH.

‡ Present address: Exxon Research and Engineering Company, Corporate Research Laboratories, P.O. Box 45, Linden, New Jersey 07036.

[1] Abbreviations and symbols used: ir, infrared; LD, linear dichroism; ⊥, perpendicular; ∥, parallel; O, unpolarized.

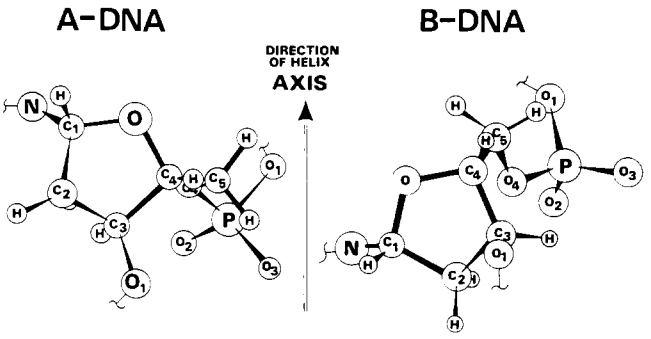

FIGURE 1: Structure of the sugar phosphate backbone of A-DNA and B-DNA. The position of C, N, P, and O atoms are from Arnott's analyses of fiber diagrams (A-DNA, Arnott and Hukins, 1972b; B-DNA, Arnott, 1974, personal communication of a refined C′(3)-endo structure similar to that published by Arnott and Hukins (1972b)). Hydrogen atoms are inserted with bond length 1.09 Å; HCH bond angle is 109°; H atoms are symmetrically arrayed with respect to the atoms joined to C.

sample, the light falls on a liquid-nitrogen-cooled, solid-state photon detector (InSb or HgCdTe). Electronic synchronous detection techniques are then used to process the electrical signal from the detector to yield a recorded plot of LD vs. wavelength. The instrument has been described in detail elsewhere (Chabay and Holzwarth, 1975).

This polarization-modulation method is about 100-fold more sensitive than classical infrared dichroism methods in which $A_\parallel$ and $A_\perp$ are separately measured. The enhanced sensitivity arises from three factors. First, since neither sample nor polarizer are moved during the measurement, the beam traverses the same regions of the film for $A_\parallel$ and $A_\perp$ measurements. Any nonuniformity of the film thickness is therefore of minor importance. Second, LD is directly measured, so solvent and unoriented chromophores are ignored. Third, the cooled solid-state detector provides better detectivity than the thermocouples conventionally used in infrared spectrophotometers.

The precision of the LD data will be apparent in Figures 2 and 3. The relative height of different peaks is accurate to about ±5%; the uncertainty arises from small errors in the automatic mechanism which adjusts the modulator to half-wave retardation at each wavelength. The absolute values of $A_\perp - A_\parallel$ given in Figures 2 and 3 were obtained by calibrating the instrument with a wire-grid polarizer in place of the polymer film. The absolute LD values thus obtained could be too large by a factor of 2.

Infrared absorption spectra $\bar{A}$ were measured with a Beckman IR-7 spectrophotometer. The data were digitized and solvent absorbance was subtracted away by computer using solvent spectra obtained on the same spectrophotometer.

Data Analysis

From the measured isotopic absorbance $\bar{A}$ and linear dichroism $\Delta A = A_\perp - A_\parallel$ of an oriented film of DNA, one can calculate the angle θ which the transition moment makes with the helix axis as follows. First, we must determine the degree of orientation of the sample. We use the model of Fraser (1958) and treat the system as having a fraction of the polymer, f, that is perfectly oriented and the remainder, $1 - f$, as being randomly oriented; the effects of alternative models are discussed by Fraser and McRae (1973). We then choose a transition for which θ is known approximately and evaluate f, using the observed ΔA and $\bar{A}$ data for this band, from the relation (Fraser, 1958):

$$1/f = 1 + 3(R \sin^2 \theta - 2 \cos^2 \theta)/2(1 - R) \qquad (1)$$

where R is the observed dichroic ratio for the partially oriented film at this transition:

$$R = (\bar{A} - (\Delta A/2))/(\bar{A} + (\Delta A/2)) = A_\parallel/A_\perp \qquad (2)$$

Following most earlier workers, we use the intensely dichroic band of deuterated DNA at 1690 cm^{-1} to estimate f for our DNA films. ^{18}O substitution (Miles, 1964) and normal coordinate analysis (Tsuboi et al., 1973) show that this band arises largely from $C_2{=}O$ stretching motions in thymine; these facts, plus infrared dichroism measurements on a hydrogen-bonded adenine-1-methylthymine single crystal (Kyogoku et al., 1967), show that the transition moment of this band is polarized along the thymine $C_2{=}O$ bond direction.

It happens that the C_2–O bond direction of thymine is nearly

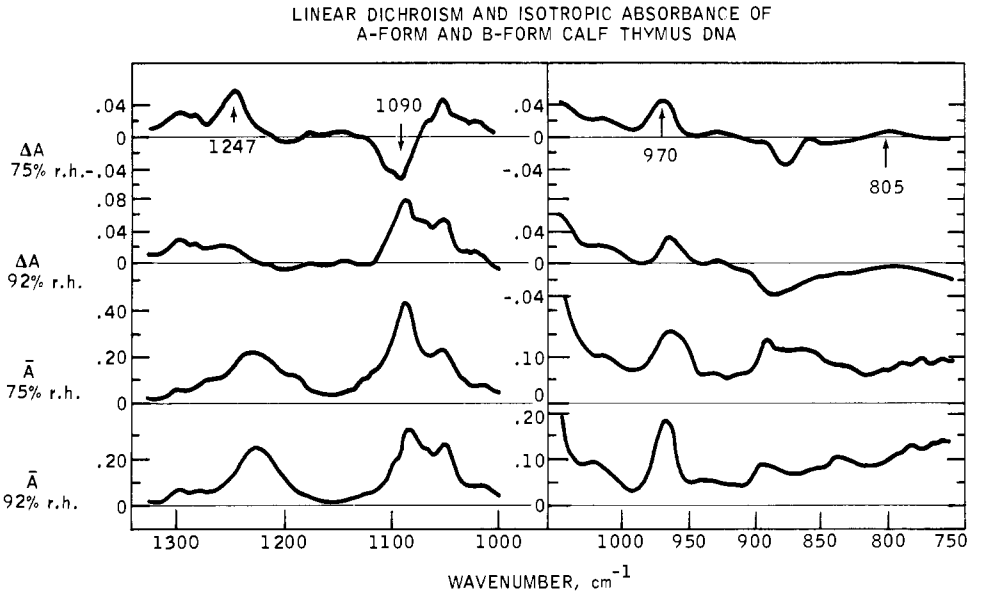

FIGURE 2: Linear dichroism and isotropic absorption spectra of a DNA film at 75 and 92% relative humidity (H_2O). Absorbance due to water has been subtracted. The same film was used for all four curves.

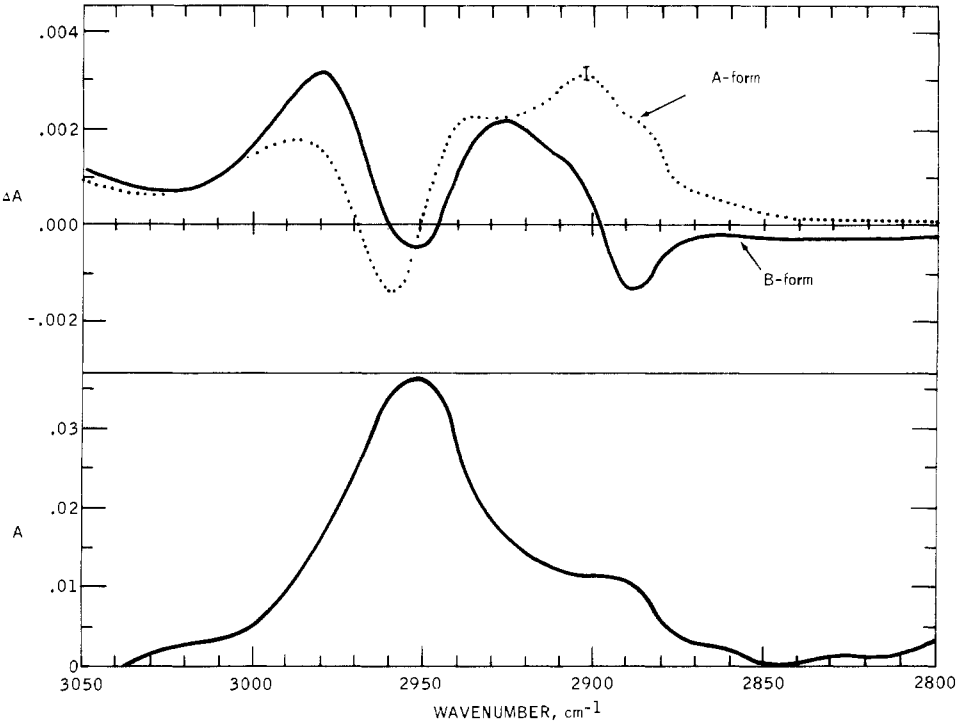

FIGURE 3: (Upper frame) Linear dichroism $\Delta A = A_{\perp} - A_{\parallel}$ for calf-thymus DNA film. A form was obtained at 75% relative humidity (D_2O). B form was obtained at 92% relative humidity. (D_2O). The same film was used for these two curves. The error bar marks the instrumental noise level. (Lower frame) Absorption spectrum of calf-thymus DNA film above. The spectrum presented is a composite: the region 2800-2934 cm^{-1} was measured in 100% relative humidity (H_2O); the region 2936-3050 cm^{-1} was measured in 100% relative humidity D_2O. The absorption due to liquid-phase H_2O has been subtracted from the 2800-2934 region.

perpendicular to the axis of tilt of the thymine base. As a consequence, the bond direction makes an angle of 88° with the helix axis in Arnott's A-form model (Arnott and Hukins, 1972b), in spite of the 20° angle between the base normal and the helix axis. The C_2-O angle is 85-89° for the B models (Arnott and Hukins, 1972b; Arnott, 1974). We therefore choose $\theta = 87.5 \pm 2.5°$ for B form and $\theta = 85 \pm 5°$ for A-DNA. Given the value of θ for the 1690 cm^{-1} bond, we calculate the value of f using eq 1 and 2 and measured values of A and ΔA. Once f is known for a particular sample, we can calculate for other absorption bands the dichroic ratio R_0 which would have been observed if the film were completely oriented (Krimm, 1960):

$$R_0 = \frac{f(R+2) + 2(R-1)}{f(R+2) - (R-1)} \quad (3)$$

The angle θ for this transition is then easily calculated:

$$\cot^2 \theta = R_0/2 \quad (4)$$

In cases where the bands overlapped, the true value of A or ΔA was obtained by fitting the sum of several Lorentzian bands to the observed curve. Convergence of the calculated curves to the observed spectra was obtained by trial and error variation in band height, width, and location.

How much error in θ is caused by our stated twofold uncertainty in instrumental calibration of ΔA? The calibration factor enters into both eq 1 and 3, because both the 1690 cm^{-1} band and the other bands are equally affected. As a consequence, the effects of the calibration factor largely cancel, and θ is not significantly altered by a twofold change in the calibration factor.

Results

In Figure 2 we show the isotropic absorption spectra and the linear dichroism spectra of A- and B-form calf thymus DNA in the region 1450 to 750 cm^{-1}. Essentially identical spectra were obtained for salmon sperm DNA (B and A forms) and for *Micrococcus luteus* DNA (B form). In general, our results for B-form linear dichroism in this spectral region agree with those of all earlier workers (Sutherland and Tsuboi, 1957; Bradbury et al., 1961; Falk et al., 1963; Pilet and Brahms, 1973; Nishimura et al., 1974). Our results for A form agree with those of Pilet and Brahms (1972, 1973) and of Nishimura et al. (1974).

Phosphate Bands. The intense absorption bands at 1230 and 1090 cm^{-1} have been previously assigned to the antisymmetric and symmetric P-O stretches, respectively, of the unesterified oxygens of the phosphate group (Tsuboi, 1957; Shimanouchi et al., 1964). The band at 1230 cm^{-1} shows weak perpendicular dichroism at 92% relative humidity (B form). The dichroism increases after heating and lowering the relative humidity to 75% (A-form DNA). In Figure 2 one can see that the dichroic band peaks at 1247 cm^{-1}. The band at 1090 cm^{-1} shows strong perpendicular dichroism for B form and parallel dichroism for A form. If one assumes that the two unesterified oxygen-phosphorus bonds are identical and that the normal modes are confined to the PO_2^- group, then it follows on symmetry grounds that the symmetric O=P—O^- stretch transition moment is directed along the bisector of the O—P—O bond angle. Similarly, the transition moment for the antisymmetric stretch (1247 cm^{-1}) then lies along the O-O line. Thus, the dichroism of these two bands provides a sensitive measurement of the phosphate group orientation.

We have calculated the value of θ from the data of Figure 2 for these transitions. In Table I our results are compared with those of Pilet and Brahms (1973), also based on ir dichroism, and to angles calculated from refined molecular models based on x-ray diffraction data. The calculated angles are based on

TABLE I: Angles for Phosphate Stretching Modes.

Mode	A-DNA			B-DNA			
	Ir			Ir		X-Ray[c]	
	This Study	Pilet-Brahms[a]	X-Ray[b]	This Study	Pilet-Brahms[a]	C(3)-exo	C(2)-endo
Antisymmetric (1247 cm^{-1})	65 ± 5	62 ± 3	77 (65)	64-90	56 ± 5	75 (77)	60 (71)
Symmetric (1090 cm^{-1})	50 ± 2	48 ± 3	17 (31)	75 ± 4	69 ± 8	54 (50)	65 (57)

[a] Pilet and Brahms, 1973. [b] Arnott and Hukins, 1972b. The angles for the OPO bisector and O-O line, using the A-DNA coordinates in this reference, were incorrectly evaluated by Arnott and Hukins (1972b). Numbers in parentheses are for earlier coordinates of Arnott et al. (1969). [c] Unpublished coordinates provided by Arnott (personal communication, 1974). The numbers in parentheses are based on older, published coordinates: C′(3)-exo from Arnott and Hukins (1972b); C′(2)-endo from Arnott et al. (1969).

O=P—O^- bond directions only, not on a normal coordinate analysis of the ribose phosphate chain. The two sets of LD results are in good agreement. Our result for the 1247 cm^{-1} band in B form has a lower bound of 64°; due to overlap with the band near 1230 cm^{-1}, we can only determine an upper bound for the absorbance of the 1247 cm^{-1} band. For B form, the ir and x-ray results are in fairly good agreement. The observed strongly perpendicular dichroism of the 1090 cm^{-1} band is in better agreement with the C′(2)-endo than with the C′(3)-exo model. For the A form, the ir results and x-ray model agree only crudely, i.e., with respect to polarization.

The LD spectra (Figure 2) show a band at 805 cm^{-1} which is perpendicularly polarized in A-DNA. In B-DNA no LD appears in this region. This confirms earlier work of Nishimura et al. (1974). Studies on dimethyl phosphate (Shimanouchi et al., 1964) report a band at 810–820 cm^{-1} which is assigned to the antisymmetric phosphate ester stretch. On the other hand, Raman studies of DNA fibers show a strongly polarized band at 807 cm^{-1} in A-DNA but not in B-DNA (Peticolas, 1972). The Raman band has been assigned to the symmetric phosphate ester stretch (Small and Peticolas, 1971; Thomas et al., 1971; Thomas and Hartman, 1973; Brown and Peticolas, 1975).

An additional noteworthy feature in Figure 2 is the dichroism at 970 cm^{-1}. For the 970 cm^{-1} band, the LD data yield $\theta = 60°$ in B form, 87° in A form. The frequency and intensity of the band suggest it is the ribose C′(4)-C′(5) stretch (Tsuboi, 1969). The C′(4)-C′(5) bond in the x-ray models makes an angle of 62° in B form, 88° in A form, in excellent agreement with the ir results.

CH Stretch Bands. In Figure 3 are shown LD and absorption spectra for A- and B-DNA between 2800 and 3040 cm^{-1}. LD data for this region have not previously been published, because the linear dichroism is weak. Only seven deoxyribose and two thymine CH stretching modes are expected to show absorption in this region, for samples equilibrated with D_2O vapor. The LD spectra show a richness of structure, with bands at 2865, 2890, 2910, 2930, 2960, and 2975 cm^{-1}. At low humidity (A form), only the 2960 cm^{-1} band is parallel-polarized. However, when the humidity is raised to 92% (B form), the band at 2890 shifts to parallel polarization, and the 2975 cm^{-1} band becomes more dichroic. The absorption spectrum of a DNA film at 92% relative humidity is also shown in Figure 3. These data are less precise than the LD curves. Subtraction of H_2O absorption makes the spectral region 2950–3050 particularly uncertain.

Tentative assignment of these bands to specific CH stretching modes can be begun on the basis of frequencies observed for specific modes in simpler compounds. The 2890 cm^{-1} band is most probably a CH_2 symmetric stretch. We base this assignment on its frequency (Fox and Martin, 1940) and its relatively high absorption intensity (Bellamy, 1968). There are two CH_2 groups in DNA, at C′(2) and C′(5). We believe that the 2890 cm^{-1} band originates in C′(5)H_2 motions because LD bands appear at 2890 cm^{-1} for poly(A) films and 2896 cm^{-1} for poly(I)·poly(C) films. These polyribonucleotides lack the C′(2)H_2 group.

The 2960 cm^{-1} LD band is probably associated with the exceptionally strong absorption peak at 2950 cm^{-1}. Strong absorption at this frequency is characteristic of CH_3 stretching motions and, to a lesser extent, antisymmetric CH_2 modes (Bellamy, 1968), which could originate in thymine and deoxyribose, respectively.

In Table II we give the angle between various CH bonds and the helix axis for A- and B-DNA models. For A-DNA, all seven deoxyribose bands are expected to be perpendicular except the C′(5)H_2 antisymmetric stretch, which is predicted to be strongly parallel (6°). The only other parallel band is the thymine CH_3 antisymmetric mode, which would be expected at 2962 ± 10 cm^{-1} (Bellamy, 1968).

For B-DNA we include predictions for four different models: two of the models use a C′(3)-exo ribose ring and two use C′(2)-endo. All of the models are from Arnott's work; they represent various stages of refinement in stereochemical criteria. For all of the B models, the predicted CH polarizations differ only slightly. In comparing the predicted polarizations of A and B-DNA, however, the change at C′(5)H_2 symmetric stretch is most striking since it goes from 79–87 to 6°. The corresponding antisymmetric mode changes from 8–11 to 87°. The 90° structural flip executed by the C′(5)H_2 group, according to the x-ray work, is apparent in Figure 1. Changes also occur in predictions for other deoxyribose modes: the C′(2)H_2 antisymmetric mode shifts from perpendicular to unpolarized, while the C′(2)H_2 symmetric mode shifts from weakly perpendicular to weakly parallel. The thymine methyl modes remain essentially unchanged in the B → A transition.

We interpret the observed change in sign of the LD at 2890 cm^{-1} as strong confirmation of the structural change at C′(5) deduced from x-ray scattering. The angles estimated from the 2890 cm^{-1} band are 63° in A form and 44° in B form; the observed dichroism is thus less extreme than that predicted from the x-ray work. The angles calculated for the ir results are uncertain because of the very weak absorbance. Conformational heterogeneity may also be a factor.

The other bands are at this point not assignable with any confidence. Normal coordinate analysis of glucose (Vasko et al., 1972) shows that the 2900 cm^{-1} bands arise from essentially pure CH stretching motions. However, the normal

TABLE II: Polarization of CH Stretching Modes Predicted from Coordinates Based on X-Ray Diffraction.[a]

Group	Bond	A-DNA	B-DNA C′(3)-exo		B-DNA C′(2)-endo	
Deoxyribose	C′(1)H	⊥ 78	⊥ 62	⊥ 66	⊥ 71	⊥ 79
	C′(2)H_2 sym	⊥ 66	∥ 45	∣ 33	∥ 34	∥ 32
	anti	⊥ 82	∥ 48	O 57	O 56	O 59
	C′(3)H	⊥ 80	⊥ 86	⊥ 87	⊥ 81	⊥ 78
	C′(4)H	⊥ 83	O 56	O 55	O 58	⊥ 61
	C′(5)H_2 sym	⊥ 87	∥ 10	∣ 8	∥ 13	∥ 11
	anti	∥ 6	⊥ 81	⊥ 87	⊥ 79	⊥ 82
Thymine	CH_3 sym	⊥ 89		⊥ 89		⊥ 85
	anti	∥ 45[b]		∣ 45[b]		∥ 45[b]
Coordinate Ref		*c*	*c*	*d*	*e*	*d*

[a] Angles listed are degrees between bond and helix axis. [b] Angle giving the expected dichroism according to eq 4. The actual angle is confined to a plane perpendicular to the thymine C_5–CH_3 bond direction. [c] Arnott and Hukins (1972b). [d] S. Arnott (personal communication, 1974). [e] Arnott et al. (1969).

coordinate work also suggests that the various CH motions may be coupled to one another (J. L. Koenig, private communication). Studies with deuterated model compounds, more precise absorption spectra, and a normal coordinate analysis of the deoxyribose–phosphate chain would allow further interpretation of the LD data we report here.

Discussion

The data presented in Figures 2 and 3 show that the B → A transition leads to profound changes in the orientation of several vibrational transition moments. The foolproof structural interpretation of such data requires that the degree and type of orientation of the film be known, that the normal modes responsible be identified, and that the transition moment directions of the normal modes be understood. In the case of DNA, the orientation can be established because the bases provide a reliable internal reference. A normal-mode analysis for a portion of the deoxyribose–phosphate chain in A and B geometry was recently carried out by Brown and Peticolas (1975). Tsuboi's work on phosphate esters (Tsuboi, 1957; Shimanouchi et al., 1964) also provides guidance on phosphate modes. Normal coordinates of ribose have not been calculated.

In the case of DNA interchain distances are large and the molecule is structurally complex, so that coupling between chains can probably be safely neglected. Coupling between nucleotides in the same chain may occur, however, and coupling between degenerate or nearly degenerate modes within a nucleotide can be expected to be strong. We have considered only the symmetric and antisymmetric modes of each CH_2 group in our analysis; coupling among CH modes on different carbons has been ignored. For C′(5)H_2 this is probably satisfactory since the group in question is the only one not in the deoxyribose ring.

The direction of a transition moment with respect to bond directions is not easily established in all cases since both nuclei and electrons contribute to the moment. For the CH transitions one can be confident that the transition moment is accurately given by a normal coordinate analysis, but for the phosphate modes this is less certain. In particular, we worry that the two unesterified oxygen atoms attached to phosphorus in DNA reside in somewhat distinct environments. This could unbalance the tautomerism O=P—O^- ⇌ $^-$O—P=O and thus the transition moment direction, for the phosphate stretching modes. Moreover, water is known to have a profound effect on phsophate stretching frequencies (Shimanouchi et al., 1964; Brown and Peticolas, 1975). The hydration of A-DNA differs from that of B-DNA.

A normal coordinate analysis carried out by Brown and Peticolas (1975) for the C′(3)C′(4)C′(5)OP(=O)(O^-)O C′(3)C′(4)C′(5) portion of the deoxyribose–phosphate chain of A- and B-DNA shows the extent of localization of the phosphate modes. The potential energy contributions of the PO_2^- stretch to the 1247 cm^{-1} band are high: 84 and 83% in A- and B-DNA, respectively. For the 1090 band, however, the normal coordinate analysis shows only 62 and 56% of the potential energy in the PO_2^- stretching motions. It is possible that the delocalization of the motions of this mode contributes to the especially sharp disagreement seen at 1090 cm^{-1} (Table I) between our LD observations and the simple predictions based on the x-ray PO_2^- bond directions for A-DNA. Incomplete conversion of the film to A-form could also contribute to the disagreement.

For the CH modes, the data we have presented support the structural flip at C′(5) seen in the x-ray model. Further high precision LD studies of polynucleotides, including homopolymers and a normal coordinate analysis, would provide a valuable check on the x-ray work. Resolution of the C′(3)-exo vs. C′(2)-endo controversy by more careful study of the CH modes will be difficult since Table II shows only small predicted differences between the two choices.

Acknowledgment

We are grateful to Professor J. Koenig for sending us unpublished details from his normal coordinate analysis of glucose, to Professor S. Arnott for precise coordinates of various DNA models, and to Dr. Su-yun Chung for her help in preparing DNA films. Computation facilities were kindly made available by Exxon Research and Engineering Company.

References

Arnott, S. (1970), *Prog. Biophys. Mol. Biol. 21*, 265.

Arnott, S. (1974), personal communication. Professor Arnott has kindly provided us with unpublished C(3)-exo and C(2)-endo coordinates stereochemically more satisfactory than those published in Arnott and Hukins (1972b) and Arnott et al. (1969).

Arnott, S., Dover, S. D., and Wonacott, A. J. (1969), *Acta Crystallogr. Sect. B 25,* 2192–2206.
Arnott, S., and Hukins, D. W. L. (1972a), *Biochem. J. 130,* 453–465.
Arnott, S., and Hukins, D. W. L. (1972b), *Biochem. Biophys. Res. Commun. 47,* 1504–1509.
Arnott, S., and Hukins, D. W. L. (1973), *J. Mol. Biol. 81,* 93–105.
Bellamy, L. J. (1968), Advances in Infrared Group Frequencies, London, Methuen.
Bradbury, E. M., Price, W. C., and Wilkinson, G. R. (1961), *J. Mol. Biol. 3,* 301.
Brown, E. B., and Peticolas, W. L. (1975), *Biopolymers 14,* 1259–1271.
Chabay, I., and Holzwarth, G. (1975), *Appl. Opt. 14,* 454–459.
Champeil, Ph., Tran., T. P. L., and Brahms, J. (1973), *Biochem. Biophys. Res. Commun. 55,* 881–887.
Falk, M., Hartman, K. A., and Lord, R. C. (1963), *J. Am. Chem. Soc. 85,* 391.
Fox, J. J., and Martin, A. E. (1940), *Proc. R. Soc. London, Ser. A 175,* 208. Bellamy misquotes this reference as to symmetric–antisymmetric assignment.
Franklin, R. F., and Gosling, R. G. (1953), *Acta Crystallogr. 6,* 673.
Fraser, M. J., and Fraser, R. D. B. (1951), *Nature (London) 167,* 759.
Fraser, R. D. B. (1958), *J. Chem. Phys. 28,* 1113.
Fraser, R. D. B., and McRae, T. P. (1973), Conformation in Fibrous Proteins, New York, N.Y., Academic Press.
Hartman, K. A., Lord, R. C., and Thomas, G. J., Jr. (1973), in Physico-Chemical Properties of Nucleic Acids, Vol. 2, Duchesne, J., Ed., New York, N.Y., Academic Press, pp 1–89.
Krimm, S. (1960), *Fortschr. Hochpolym.-Forsch. 2,* 51–172.
Kupke, I., and Sauer, D. (1970), *Z. Anal. Chem. 250,* 101–104.
Kyogoku, Y., and Higuchi, S., and Tsuboi, M. (1967), *Spectrochim. Acta, Part A 23,* 969.
Miles, H. T. (1964), *Proc. Natl. Acad. Sci. U.S.A. 51,* 1104.
Nishimura, Y., Morikawa, K., and Tsuboi, M. (1974), *Bull. Chem. Soc. Jpn. 47,* 1043–1044.
Peticolas, W. L. (1972), *Adv. Raman Spectrosc. 1,* 285–295.
Pilet, J., Blicharski, J., and Brahms, J. (1975), *Biochemistry 14,* 1869–1876.
Pilet, J., and Brahms, J. (1972), *Nature (London), New Biol. 236,* 99–101.
Pilet, J., and Brahms, J. (1973), *Biopolymers 12,* 387–403.
Rupprecht, A., and Forslind, B. (1970), *Biochem. Biophys. Acta 20,* 304.
Shimanouchi, T., Tsuboi, M., and Kyogoku, Y. (1964), *Adv. Chem. Phys. 7,* 435.
Small, E. W., and Peticolas, W. L. (1971), *Biopolymers 10,* 1377.
Sutherland, G. B. B. M., and Tsuboi, M. (1957), *Proc. R. Soc. London, Ser. A 239,* 446–463.
Thomas, G. J., and Hartman, K. A. (1973), *Biochim. Biophys. Acta 312,* 311–322.
Thomas, G. J., Jr., Medeiros, G. D., and Hartman, K. A. (1971), *Biochem. Biophys. Res. Commun. 44,* 587.
Tsuboi, M. (1957), *J. Am. Chem. Soc. 79,* 1351.
Tsuboi, M. (1969), *Appl. Spectrosc. Rev. 3,* 45–90.
Tsuboi, M., Takahasi, S., and Harada, I. (1973), in Physico-Chemical Properties of Nucleic Acids, Vol. 2, Duchesne, J., Ed., New York, N.Y., Academic Press, pp 92–145.
Vasko, P. D., Blackwell, J., and Koenig, J. L. (1972), *Carbohydr. Res. 23,* 407–416.