INTRINSIC BIREFRINGENCE OF MULTIPLE-COILED DNA, THEORY AND APPLICATIONS

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ABSTRACT The intrinsic birefringence of multiple-coiled DNA is computed in terms of an equally dense array of parallel DNA molecules. The birefringence for *n* times–coiled DNA molecules is given by

$$\Gamma_n = (-\tfrac{1}{2})^{n-1}\left\{\prod_{i=0}^{n-1} (1 - 3\sin^2 \beta_i)\right\}\Gamma_{\mathrm{DNA}}$$

where

$\beta_0 = 0$, $\beta_i = \tan^{-1} (p_i/2\pi r_i)$, p_i = period of the i^{th} helix coil and r_i = radius of i^{th} helix coil. The formula is applied to two cases of helically coiled DNA in biological material and found to agree quantitatively with experimental results.

INTRODUCTION

The optical properties of DNA have been extensively studied as a means to elucidate the structure of the molecule. Ultraviolet dichroism and birefringence were measured on oriented sheets (1) and fibers of DNA at different degrees of humidity (2, 3). The birefringence and ultraviolet dichroism (4) and infrared dichroism (5) are negative. For fiber bundles of materials whose dielectric constant differs from the surrounding material, it has been shown (6) that the form birefringence and dichroism would be positive for small fractional volumes of the fibers. Bruggemann (7) proved the general case for all volume fractions between 0 and 1. Therefore the form birefringence of DNA fibers is positive. However, the observed strong negative birefringence ($\Gamma = -0.11$) (4) and dichroism of DNA fibers points out that the main cause for the anisotropies in propagation velocity and absorptivity is due to the orientation of the purine and pyrimidine bases in the molecule (4). These bases have been shown to be perpendicular to the main axis of the molecule (8) in the B form of DNA, the type that is found in conditions of very high humidity and in aqueous solutions (9). In the following calculations the intrinsic birefringence of DNA is attributed solely to the orientation of the base planes. The array of DNA molecules will be treated as a loseless dielectric composed of a collection of base pairs. The positions of the planes of the bases are determined by the geometry of the coiling. Parallel and perpendicular polarizabilities are defined for each base plane. The indices of refraction will be expressed in terms of induced polarization densities of this dielectric as it is polarized by the electric vector **E** of the light wave.

Birefringence of a Fiber of Parallel DNA Molecules. Consider a parallel array of DNA molecules oriented in the direction of an arbitrary Z axis. The base planes lie all parallel to the xy plane of the coordinate system (Fig. 1). This array of bases interacts with two plane polarized light waves, one with its electric vector parallel to the Z axis ($\mathbf{E}_z$) and the other with its electric vector parallel to the xy plane say, ($\mathbf{E}_x$).

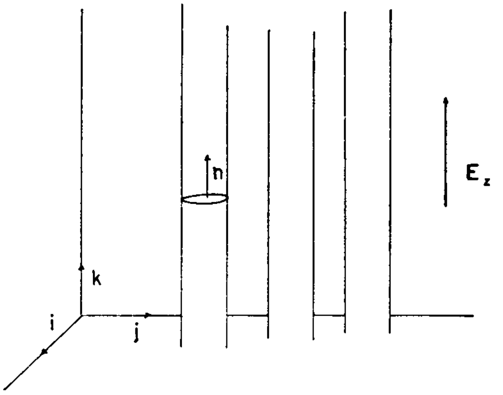

FIGURE 1 Array of DNA molecules parallel to the Z axis **n** is perpendicular unit vector to the base plane of DNA molecule.

For a dielectric without losses (ϵ real) we have

$$\mathbf{D} - \mathbf{E} = 4\pi\mathbf{P}, \qquad \mathbf{D} = \epsilon\mathbf{E} \tag{1}$$

D = displacement vector
E = polarizing field
P = polarization density
ϵ = dielectric constant.

In the optical region the index of refraction n is given by

$$n^2 = \epsilon\mu = \epsilon \quad \text{since} \quad \mu \cong 1 \tag{2}$$

Therefore

$$n^2 = \epsilon = 1 + 4\pi \frac{|\mathbf{P}|}{|\mathbf{E}|} \tag{3}$$

Equation (3) is valid and can be applied only if: (Condition A) **E**, the polarizing field and **P**, the resultant polarization density, are parallel to each other and point in the same direction.

Assign to each base plane in the DNA array a normal unit vector **n**. In the DNA fiber oriented along the Z axis **n** = **k**, where **k** is a unit vector along the Z axis. For a field $\mathbf{E}$, striking a base (i^{th} base) defined by the normal vector $\mathbf{n}_i$ (x, y, z), we have induced dipoles $\mathbf{m}_{||i}$ (parallel to i^{th} base plane) and $\mathbf{m}_{\perp i}$ (perpendicular to the i^{th} base plane). These are given by

$$\mathbf{m}_{\parallel i} = \alpha_{\parallel}\mathbf{E}_{\parallel i}, \qquad \mathbf{m}_{\perp i} = \alpha_{\perp}\mathbf{E}_{\perp i} \tag{4}$$

$\mathbf{E}_{\perp i}$ = component of E perpendicular to i^{th} base
$\mathbf{E}_{||i}$ = component of E parallel to i^{th} base
$\alpha_{\perp}$ = perpendicular polarizability of base pair
$\alpha_{||}$ = parallel polarizability of base pairs.

The induced dipole for an array of parallel base planes is equal to

$$\sum_i \alpha_{\parallel}\mathbf{E}_{i\parallel} + \sum_i \alpha_{\perp}\mathbf{E}_{i\perp} \tag{5}$$

The polarization density is then

$$\frac{1}{\mathrm{V}}\left(\sum_i \alpha_{\parallel}\mathbf{E}_{\parallel i} + \sum_i \mathbf{E}_{\perp i}\alpha_{\perp}\right) = \mathbf{P} \tag{6}$$

V = volume occupied by N_0 base pairs. For the above geometry all $\mathbf{E}_{||i}$ are equal and all $\mathbf{E}_{\perp i}$ are equal so summing over a total of N_0 base pairs in volume V,

$$\frac{N_0}{\mathrm{V}}\alpha_{\parallel}\mathbf{E}_{\parallel} + \frac{N_0}{\mathrm{V}}\alpha_{\perp}\mathbf{E}_{\perp} = \mathbf{P} \tag{7}$$

where

$$\mathbf{E}_{\parallel} = \mathbf{E}_{\parallel i} \quad \text{for all} \quad i\text{'s}$$
$$\mathbf{E}_{\perp} = \mathbf{E}_{\perp i} \quad \text{for all} \quad i\text{'s}$$

Let

$N_0/V = \rho$ = density of the base pair molecules.

Define a polarizability density α' so that

$$\alpha'_{\perp} = \rho\alpha_{\perp}, \qquad \alpha'_{\parallel} = \rho\alpha_{\parallel} \tag{8}$$

and the equation (7) reduces then to

$$\alpha_{\perp}'\mathbf{E}_{\perp} + \alpha_{\parallel}'\mathbf{E}_{\parallel} = \mathbf{P} \tag{9}$$

$\mathbf{P}$ = polarization density. The parameters $\alpha_{\perp}'$, $\alpha_{||}'$ are now expressed in terms of dipole density. This is done so as to allow the computation of the total polarization in terms of volume integrals. We define $\mathbf{n}$ as a unit vector normal to the plane of a base pair. The perpendicular and parallel projections of vector $\mathbf{E}$ to the plane are given by

$$\begin{aligned} \mathbf{E}_{\perp} &= (\mathbf{E}\cdot\mathbf{n})\mathbf{n} \\ \mathbf{E}_{\parallel} &= (\mathbf{n} \times \mathbf{E}) \times \mathbf{n} \end{aligned} \tag{10}$$

For $\mathbf{E} = E_x\mathbf{i}$ and $\mathbf{n} = \mathbf{k}$ (see Fig. 1)
we have

$$\begin{aligned} \mathbf{E}_\perp &= (E_x \mathbf{i}\cdot\mathbf{k})\mathbf{k} = 0 \\ \mathbf{E}_\parallel &= (\mathbf{k} \times \mathbf{i}E_x) \times \mathbf{k} = E_x\mathbf{i} \end{aligned} \tag{11}$$

and $\mathbf{P} = \alpha_{\parallel}' E_x\mathbf{i}$ or $\mathbf{P}\|\mathbf{E}$
i. e., Condition A is satisfied. Therefore for a DNA molecule oriented parallel to the Z axis, the index of refraction along the x direction is given by equation (3)

$$(n_{\mathrm{DNA},x})^2 = 1 + 4\pi \frac{|\mathbf{P}|}{|\mathbf{E}|} = 1 + 4\pi\alpha_\parallel' \tag{12}$$

Similarly if $\mathbf{E} = \mathbf{E}_z\mathbf{k}$ then $\mathbf{E}_\perp = (\mathbf{n} \cdot \mathbf{E})\mathbf{n} = E_z\mathbf{k}$

$$\mathbf{E}_\parallel = (\mathbf{n} \times \mathbf{E}) \times \mathbf{n} = (E_z\mathbf{k} \times \mathbf{k}) \times \mathbf{k} = 0 \tag{13}$$

and

$$\mathbf{P} = \alpha_\perp' E_z\mathbf{k} = \alpha_\perp'\mathbf{E} \tag{14}$$

Equation (14) satisfies Condition A. Therefore

$$(n_{\mathrm{DNA},z})^2 = 1 + 4\pi\alpha_\perp' \text{ where}$$

$n_{\mathrm{DNA},z}$ = index of refraction of DNA molecule along its main axis. Notice that equations (12) and (14) involve the *squares* of the indices of refraction.

The Birefringence of Coiled DNA (secondary Helix). The DNA molecule (the Watson-Crick double helix) is coiled into a secondary helix with pitch p_1 and radius r_1. The equation of the helix in terms of the parameter θ_1 (Fig. 2) is

$$\begin{aligned} x &= r_1 \cos\,\theta_1 \\ y &= r_1 \sin\,\theta_1 \\ z &= \frac{p_1}{2\pi}\,\theta_1 \end{aligned} \tag{15}$$

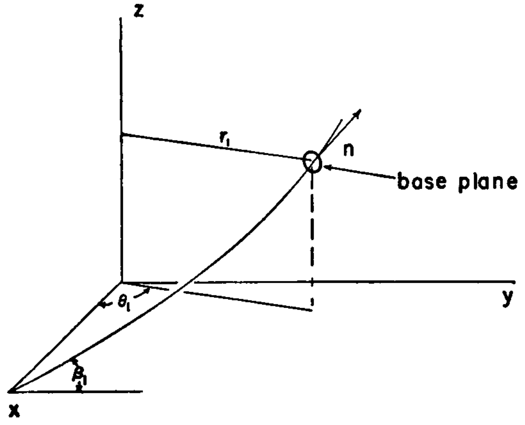

FIGURE 2 DNA molecule is coiled in a secondary helix with pitch angle β_1 and radius r_1. The axis of secondary helix is along the Z axis.

The angle of the helix with respect to the *xy* plane is given by

$$\tan \beta_1 = \frac{p_1}{2\pi r_1}$$

and

$$z = \frac{p_1}{2\pi}\,\theta_1 = r_1(\tan \beta_1)\theta_1 \tag{16}$$

The normal to the base plane for this case would be

$$\mathbf{n} = -\cos \beta_1 \sin \theta_1 \mathbf{i} + \cos \beta_1 \cos \theta_1 \mathbf{j} + \sin \beta_1 \mathbf{k} \tag{17}$$

We use equation (10) to determine $\mathbf{E}_\perp$ and $\mathbf{E}_{\parallel}$. Using polarizability densities $\alpha_\perp'$, $\alpha_\parallel'$ we have that

$$\mathbf{P} = \frac{1}{V}\left(\int \alpha_\perp' \mathbf{E}_\perp \, dv + \int \alpha_\parallel' \mathbf{E}_\parallel \, dv\right) \tag{18}$$

V = total volume of DNA molecule.

Detailed computations are given in Appendix I. For a secondary helix we obtain

$$\begin{aligned} n^2_{2,x} &= 1 + 4\pi\left[\frac{\alpha_\perp' \cos^2 \beta_1 + \alpha_\parallel'(1 + \sin^2 \beta_1)}{2}\right] \\ &= 1 + 4\pi(\alpha_2'{}_{,\parallel}) \end{aligned} \tag{19}$$

where

$\alpha_2'{}_{,\parallel}$ = polarizability density parallel to *xy* plane of secondary helix (Fig. 2)

and

$$\begin{aligned} n^2_{2,z} &= 1 + 4\pi(\alpha_\perp' \sin^2 \beta_1 + \alpha_\parallel' \cos^2 \beta_1) \\ &= 1 + 4\pi(\alpha_2'{}_{,\perp}) \end{aligned} \tag{20}$$

where $\alpha_2'{}_{,\perp}$ = polarizability density along the main axis of secondary helix. Using equations (12) and (14) we have

$$\begin{aligned} n^2_{\mathrm{DNA},z} - n^2_{\mathrm{DNA},x} &= 1 + 4\pi\alpha_\perp' - (1 + 4\pi\alpha_\parallel') \\ &= 4\pi(\alpha_\perp' - \alpha_\parallel') \end{aligned} \tag{21}$$

Equation (19) and (20) give

$$\begin{aligned} n^2_{2,z} - n^2_{2,x} &= 1 + 4\pi(\alpha_\perp' \sin^2 \beta_1 + \alpha_\parallel' \cos^2 \beta_1) \\ &\qquad - \left[1 + 4\pi\left\{\frac{\alpha_\perp' \cos^2 \beta_1 + \alpha_\parallel'(1 + \sin^2 \beta_1)}{2}\right\}\right] \\ &= -2\pi(\alpha_\perp' - \alpha_\parallel')(1 - 3\sin^2 \beta_1) \end{aligned} \tag{22}$$

Taking the ratio of equations (22) and (21) we have

$$\frac{n^2_{2,z} - n^2_{2,x}}{n^2_{\mathrm{DNA},z} - n^2_{\mathrm{DNA},x}} = -\tfrac{1}{2}(1 - 3\sin^2 \beta_1) \tag{23}$$

but

$$\frac{n^2_{2,s} - n^2_{2,z}}{n^2_{DNA,s} - n^2_{DNA,z}} = \frac{n_{2,s} + n_{2,z}}{n_{DNA,s} + n_{DNA,z}} \cdot \frac{n_{2,s} - n_{2,z}}{n_{DNA,s} - {}_{DNA,z}} \tag{24}$$

and

$$\frac{n_{2,s} + n_{2,z}}{n_{DNA,s} + n_{DNA,z}} \cong 1 \quad \text{for} \quad 1 \leq n_{DNA,z} \leq 2 \tag{25}$$

and $n_{2,s} - n_{2,z} = \Gamma_2 =$ birefringence of secondary helix

$n_{DNA,s} - n_{DNA,z} = \Gamma_{DNA} =$ birefringence of DNA,

therefore

$$\frac{\Gamma_2}{\Gamma_{DNA}} = -\tfrac{1}{2}(1 - 3 \sin^2 \beta_1) \tag{26}$$

If $\beta_1 = 90°$, the secondary helix reduces to a parallel array of DNA molecules. We have then

$$\Gamma_2 = -\tfrac{1}{2}(1 - 3)\Gamma_{DNA} = \Gamma_{DNA}$$

The Birefringence of a tertiary Coil of DNA. A secondary coil of DNA as described in the previous section is in turn wound up in a helix so that the main axis of the secondary coil is described by equation (27). (See Fig. 3) We have

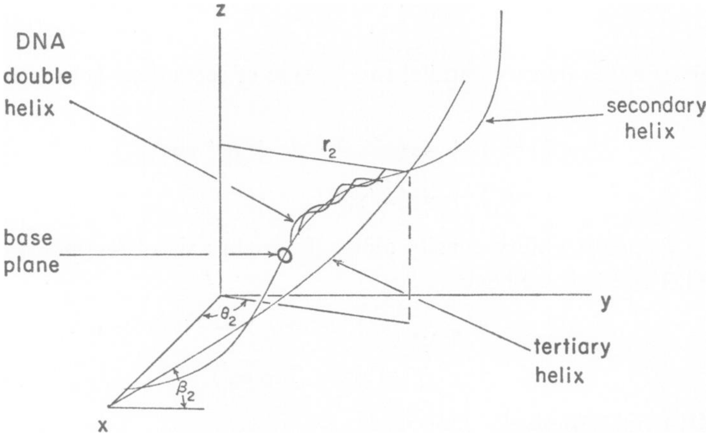

FIGURE 3 Secondary helix of DNA is coiled into a tertiary helix of pitch angle β_2 with its main axis along the Z axis.

$$\begin{aligned} x' &= r_2 \cos \theta_2 \\ y' &= r_2 \sin \theta_2 \\ z' &= \frac{p_2}{2\pi} \theta_2 \end{aligned} \tag{27}$$

where $p_2 =$ is period of tertiary helix and

$$\tan \beta_2 = \frac{p_2}{2\pi r_2} \tag{28}$$

β_2 = angle with respect to $x'\ y'$ plane
r_2 = radius of tertiary helix.

The indices of refraction are again computed in an analogous fashion as for the secondary helix. The normal to the base planes is now a function of four parameters (θ_1, θ_2, β_1, β_2). The first two are integration variables. β_1, β_2 are parameters depending on the periods p_1, p_2 and radius r_1, r_2 of the secondary and tertiary helices respectively and are characteristic pitch angles of any specific coiling. Detailed computations are given in Appendix II.

The index of refraction of the tertiary helix is computed to be

$$\Gamma_{\text{tertiary helix}} = \Gamma_3 = \tfrac{1}{4}(1 - 3\sin^2 \beta_1)(1 - 3\sin^2 \beta_2)\Gamma_{\text{DNA}} \tag{29}$$

If either β_1 or β_2 are equal to 90°, the tertiary helix reduces to a secondary helix. If $\beta_1 = \beta_2 = 90°$, the case reduces to a parallel array of DNA molecules again.

The Birefringence of a Multiple Helix of DNA. Let us define an n^{th} helix by the equation

$$\begin{aligned} x^{(n)} &= r_n \cos \theta_n \\ y^{(n)} &= r_n \sin \theta_n \\ z^{(n)} &= \frac{p_n}{2\pi} \theta_n \end{aligned} \tag{30}$$

where $x^{(n)}$, $y^{(n)}$, $z^{(n)}$ are coordinates in the n-primed system and r_n, p_n are the radius and period of the n-tuple helix of DNA. Also the relation

$$\tan \beta_n = \frac{p_n}{2\pi r_n} \tag{31}$$

holds. This n^{th} helix is made up of a $(n - 1)^{th}$ helix with equation

$$\begin{aligned} x^{(n-1)} &= r_{n-1} \cos \theta_{n-1} \\ y^{(n-1)} &= r_{n-1} \sin \theta_{n-1} \\ z^{(n-1)} &= \frac{p_{n-1}}{2\pi r_{n-1}} \theta_{n-1} \end{aligned} \tag{32}$$

with

$$\tan \beta_{n-1} = \frac{p_{n-1}}{2\pi r_{n-1}} \tag{33}$$

Using the birefringence of $(n - 1)^{th}$ helix, we can calculate the birefringence of n^{th} helix by analogous procedure as the calculation of the birefringence of a tertiary helix from the secondary helix. Therefore the equation for the birefringence of the n^{th} helix in terms of birefringence of the DNA molecule is given by

$$\Gamma_n = n_{n,s} - n_{n,z} = \left(-\tfrac{1}{2}\right)^{n-1} \left[\prod_{i=0}^{n-1} (1 - 3\sin^2 \beta_i)\right] \Gamma_{\mathrm{DNA}} \tag{34}$$

$$\beta_0 = 0, \qquad \tan \beta_i = \frac{p_i}{2\pi r_i}, \qquad i \geq 1 \tag{35}$$

p_i = pitch of i^{th} helix
r_i = radius of i^{th} helix

Thus we have

$$\Gamma_1 = \Gamma_{\mathrm{DNA}}$$
$$\Gamma_2 = -\tfrac{1}{2}(1 - 3\sin^2 \beta_1)\Gamma_{\mathrm{DNA}}$$
$$\Gamma_3 = \tfrac{1}{4}(1 - 3\sin^2 \beta_1)(1 - 3\sin^2 \beta_2)\Gamma_{\mathrm{DNA}}$$

etc.

The derivation of the formula for the birefringence of coiled DNA may be also performed by use of symmetry arguments.

Application of Theory to Experimental Data and to Biological Models. Studies of the birefringence of streaked pellets of T–2 bacteriophage by Bendet *et al.* (10) showed that some of the DNA of the phage was oriented parallel to its main axis. Quantitative measurements of streaming birefringence by Gellert (11) pointed out that the phage behaved as if only 9 to 16 per cent of its DNA is oriented parallel to the phage main axis, the remaining DNA being oriented randomly (16). On the basis of available experimental data obtained by different physical means (12, 13) Kilkson and Maestre (14), proposed a detailed model for the arrangement of DNA in T–2 phage. The following calculation shows that the birefringence of Kilkson-Maestre model agrees quantitatively with Gellert's (11) experimental data.

The model involves the coiling of a DNA molecule of 520,000 A in length into a secondary helix of radius $r_1 = 27$ A. This gives a total outside diameter of 76.7 A. Due to interdigitation between adjacent loops of DNA, the repeat distance is $p_1 = 20$ A. By using equation (16), an angle $\beta_1 = 6.72°$ for the secondary helix is obtained. For this value

$$1 - 3\sin^2 \beta_1 = 0.96 \tag{36}$$

The secondary helix is then wound up into four concentric tertiary helices with respectively increasing radii. Let R_1 be the longest radius and R_4 the smallest one. From the model it follows that 32.8 per cent of the mass of the molecule is at R_1, 29.6 per cent at R_2, 23.5 per cent at R_3 and 14.1 per cent at R_4. Equation (28) gives values for $\beta_{2,i}$ corresponding to each one of the concentric tertiary helices. The value of $\beta_{2,i}$ is determined by R_i and p_2. The value of p_2 is equal to the diameter of the secondary helix and is the same for all tertiary helices. Therefore the term involving β_2 in equation (29) is given by

$$(1 - 3\sin^2 \beta_2) = \left(1 - 3\sum_{i=1}^{4} f_i \sin^2 \beta_{2,i}\right)$$

where f_i is the fraction of mass in the tertiary helix at radius R_i.

For our model of the T–2 phage coiling, this term has a value of 0.9. Therefore the intrinsic birefringence of the tertiary coil packing for T–2 DNA is

$$\Gamma_{\text{tertiary coil model}} = \tfrac{1}{4}(1 - 3\sin^2\beta_1)\left(1 - 3\sum_{i=1}^{4} f_i \sin^2\beta_{2,i}\right)\Gamma_{\text{DNA}}$$

$$\Gamma_3 = \tfrac{1}{4}(0.96)(0.9)\Gamma_{\text{DNA}}$$

$$= (0.216)\Gamma_{\text{DNA}}$$

In other words the intrinsic birefringence of the tertiary coil model behaves as if only 21.6 per cent of the DNA is aligned parallel to the axis of the phage, the rest being oriented randomly.

The influence of form birefringence of a parallel array of bacteriophage is a conjectural matter since the exact form birefringence for the complicated shape of bacteriophage T–2 has not been determined. However the form birefringence of the protein coat of the bacteriophage was measured by Gellert (11) by assuming that it was the same as that of the phage ghosts. This value was used by him to compute the intrinsic birefringence of the DNA in the head of the phage. Comparing the obtained results with the values of the birefringence for aligned DNA molecules, Gellert (11, 16) arrived at a 9 to 16 per cent orientation for DNA in T–2 phage. In view of the uncertainties in computing the form birefringence effect upon the total birefringence of the phage, it can be seen that there is good agreement between the values obtained by Gellert (11) and the computed value of 21.6 per cent for the tertiary model.

Application of the Tertiary Helix Model to the DNA Organization in the Cave Cricket Sperm Head. The organization of DNA in single sperm head of the cave cricket (*Authophilus nigricans*) was experimentally studied by Inoue and Sato (15) with a high resolution polarizing microscope. They found the optical axis of the sperm head to zig zag regularly as though microcrystalline domains were oriented in the form of a helix. The variation of the azimuth angle of the microdomains had a period of 0.2 to 0.3μ along the length of the sperm head. The corresponding value of the variation of the azimuth angle was -8 to $+8°$. They irradiated these periodic domains with ultraviolet light polarized at an angle of 45° to the sperm axis. It was found that each microdomain responded to the irradiation at a different rate depending upon the azimuth orientation with respect to the main axis of the sperm head. Inoue's and Sato's interpretation is that the microdomains that contain molecules whose bases lie parallel to the E vector, absorb more ultraviolet light and lose birefringence faster than those microdomains whose bases are perpendicular to the plane of polarization. They concluded that a rotation of the average optical axis of the sperm head away from the sperm axis and towards the direction of the E vector should be observed. This was measured experimentally to be 15° away from the axis of the sperm head. They obtained an average birefringence of $\Gamma_{\text{sperm}} = -0.02$ for the sperm head (17). From the above experimental results, Inoue and

Sato concluded that the 20 to 30 A thick DNA and protein complex in the head of the sperm is coiled into a secondary helix of 2,000 A diameter. Subsequently this is coiled into a tertiary helix of 8,000 A in diameter (15). Two of these helices are intertwined together with some matrix into a quaternary helix making up the sperm chromosome. Since the Inoue and Sato model is a multiple coiled DNA, the theory of DNA birefringence (fourth section) is directly applicable. Thus from equation (34) we have

$$\frac{\Gamma_{\text{sperm head}}}{\Gamma_{\text{DNA}}} = -\tfrac{1}{8}(1 - 3\sin^2\beta_1)(1 - 3\sin^2\beta_2)(1 - 3\sin^2\beta_3) \tag{37}$$

Since the period of the quaternary helix is very large, we have that $\beta_3 = 90°$, $1 - 3\sin^2 90° = -2$ and equation (37) reduces to

$$\frac{\Gamma_{\text{sperm head}}}{\Gamma_{\text{DNA}}} = \tfrac{1}{4}(1 - 3\sin^2\beta_1)(1 - 3\sin^2\beta_2) \tag{38}$$

Therefore the structure behaves optically as a set of two parallel tertiary helices. It remains to calculate the values of β_1, β_2 from the total birefringence of the sperm head and its behavior when irradiated by the methods of Inoue and Sato. From the Inoue-Sato model

$$r_2 = 2{,}000\ \text{A}$$

$$p_2 = 2{,}000\ \text{A}$$

This gives a pitch angle β_2.

$$\tan\beta_2 = \frac{p_2}{2\pi r_2} = \frac{1}{2\pi}$$

which gives a value of $\beta_2 = 9°$. This value is in close agreement with the maximum azimuth angle of microdomains measured by Inoue and Sato:
$\beta_2(\text{experimental}) = 8°$. If we assume $\beta_2 = 9°$ and use equation (35), we have

$$\frac{\Gamma_{\text{cave cricket sperm}}}{\Gamma_{\text{DNA}}} = \frac{-0.02}{-0.11} = \tfrac{1}{4}(1 - 3\sin^2\beta_1)(1 - 3\sin^2\beta_2)$$

therefore

$$1 - 3\sin^2\beta_1 = 0.785, \qquad \sin^2\beta_1 = 0.07 \qquad \beta_1 \cong 15.3°$$

In other words in Fig. 3 the angle of coiling of the secondary helix is $\beta_1 \cong 15.3°$. Thus when the tertiary coils are irradiated at an angle of 45° from the main axis of the tertiary helix, the bases most perpendicular and most unaffected by the E vector are at an average angle of 15.3° from the main axis of the tertiary helix. Of course there is an oscillation of 8° superimposed to this value due to the tertiary helix coiling angle of $\beta_2 = 8°$. This was observed by Inoue and Sato. Therefore we have that the azimuth variation of the optical axis of the microdomains is explained by the tertiary helix coiling ($\beta_2 = 8°$). The rotation of 15° from the optical axis after ultraviolet irradiation is explained by the pitch angle of the secondary helix

($\beta_1 = 15.3°$). Therefore the application of the theory of coiled DNA birefringence to the Inoue-Sato model of sperm head-can fully explain the experimentally observed values.

CONCLUDING REMARKS

The theory of the birefringence of the multiple-coiled DNA is general in nature. There is an obvious extension of the theory to multiple helices of non-circular correction (for example, squashed helices as might be found in some biological systems). The importance of the theory is accentuated by the fact that the two applications of the theory refer to the DNA *in vivo.*

APPENDIX I

$\mathbf{n}$ is given by equation (17).
Equation (10) gives $\mathbf{E} = E_x\mathbf{i}$.
Therefore

$$\mathbf{E}_\perp = (\mathbf{n}\cdot\mathbf{E}_x\mathbf{i})\mathbf{n} = (\sin^2\theta\cos^2\beta\mathbf{i} - \cos^2\beta\cos\theta\sin\theta\mathbf{j} - \sin\theta\sin\beta\cos\beta\mathbf{k})\,E_x \tag{39}$$

$$\begin{aligned}\mathbf{E}_\parallel = (\mathbf{n}\times E_x\mathbf{i})\times\mathbf{n} = [(&\cos^2\theta\cos^2\beta + \sin^2\beta)\mathbf{i}\\ &+ \cos^2\beta\sin\theta\cos\theta\mathbf{j} + \sin\theta\sin\beta\cos\beta\mathbf{k}]\,E_x\end{aligned} \tag{40}$$

Volume element dv is given by

$$dv = A\left(r_1^2 + \frac{p_1^2}{4\pi^2}\right)^{1/2} d\theta = T\,d\theta \tag{41}$$

A = cross-sectional area of DNA molecule
Equation (18) reduces to

$$\begin{aligned}\mathbf{P} &= \frac{1}{\int_0^{2\pi} T\,d\theta}\left(\int_0^{2\pi}\alpha_\perp'\mathbf{E}_\perp T\,d\theta + \int_0^{2\pi}\alpha_\parallel'\mathbf{E}_\parallel T\,d\theta\right)\\ \mathbf{P} &= \frac{1}{2\pi}\left(\int_0^{2\pi}\alpha_\perp'\mathbf{E}_\perp\,d\theta + \int_0^{2\pi}\alpha_\parallel'\mathbf{E}_\parallel\,d\theta\right)\end{aligned} \tag{42}$$

The values of $\mathbf{E}_\perp$, $\mathbf{E}_\parallel$ given by (39) and (40) are used in equation (42). The odd functions in Θ give 0 value when integrated over $0 \leq \Theta \leq 2\pi$.
We obtain for $\mathbf{P}_x$

$$\mathbf{P}_x = \left\{\frac{\alpha_\perp'\cos^2\beta + \alpha_\parallel'(1 + \sin^2\beta)}{2}\right\}\mathbf{E}_x$$

Therefore Condition A is satisfied. Using equation (3), we obtain (19). In a similar fashion equation (20) for $n_{2,s}^2$ is obtained.

APPENDIX II

The normal to the base plane, $\mathbf{N}$, is now a function of θ_1, θ_2, β_1, β_2 *i.e.*

$$\mathbf{N}(\theta_1, \theta_2, \beta_1, \beta_2) = (\cos\theta_1 \cos\beta_1 \sin\theta_2 \sin\beta_2 - \sin\theta_1 \cos\beta_1 \cos\theta_2 - \cos\beta_2 \sin\theta_2 \sin\beta_1)\mathbf{i} + (-\cos\theta_1 \cos\beta_1 \sin\beta_2 \cos\theta_2 - \sin\theta_1 \cos\beta_1 \sin\theta_2 + \sin\beta_1 \cos\beta_2 \cos\theta_2)\mathbf{j} + (\cos\theta_1 \cos\beta_1 \cos\beta_2 + \sin\beta_1 \sin\beta_2)\mathbf{k}$$

The variables of integration are Θ_1, Θ_2.
The volume element is expressed as

$$dv(\theta_1, \theta_2, \beta_1, \beta_2) = T'(\beta_1, \beta_2)\, d\theta_1\, d\theta_2.$$

Again both $E_\parallel$, $E_\perp$ have odd and even terms in Θ_1, Θ_2. The odd terms vanish upon integration over $0 \leq \Theta_1 \leq 2\pi$, $0 \leq \Theta_2 \leq 2\pi$.
Therefore

$$T' \int_0^{2\pi} \int_0^{2\pi} \alpha_\perp' E_\perp\, d\theta_1\, d\theta_2 = \pi^2 \alpha_\perp' (\cos^2\beta_1 \sin^2\beta_2 + \cos^2\beta_1 + 2\cos^2\beta_2 \sin^2\beta_1)\mathbf{i}T'E_x$$

$$T' \int_0^{2\pi} \int_0^{2\pi} \alpha_\parallel' E_\parallel\, d\theta_1\, d\theta_2 = \pi^2 \alpha_\parallel' (2\cos^2\beta_1 \cos^2\beta_2 + 4\sin^2\beta_1 \sin^2\beta_2 + 2\cos^2\beta_2 \sin^2\beta_1 + \cos^2\beta_1 \sin^2\beta_2 + \cos^2\beta_1)E_x\mathbf{i}T'$$

From equation (18)

$$\mathbf{P}_x = \frac{T' \int_0^{2\pi} \int_0^{2\pi} \alpha_\perp' \mathbf{E}_\perp + \alpha_\parallel' \mathbf{E}_\parallel\, d\theta_1\, d\theta_2}{T' \int_0^{2\pi} \int_0^{2\pi} d\theta_1\, d\theta_2} = \frac{1}{4\pi^2} [\alpha_\perp'(\cos^2\beta_1 \sin^2\beta_2 + \cos^2\beta_2 + 2\cos^2\beta_2 \sin^2\beta_1) + \alpha_\parallel'(2\cos^2\beta_1 \cos^2\beta_2 + 4\sin^2\beta_1 \sin^2\beta_2 + \cos^2\beta_1 \sin^2\beta_2 + 2\cos^2\beta_2 \sin^2\beta_1 + \cos^2\beta_1)]E_x\mathbf{i}.$$

This satisfies Condition A.
Therefore equation (3) gives

$$n_{3,x}^2 = 1 + 4\pi \frac{|\mathbf{P}_x|}{|\mathbf{E}_x|} = 1 + \pi[\alpha_\perp'(\cos^2\beta_1 \sin^2\beta_2 + \cos^2\beta_1 + 2\cos^2\beta_2 \sin^2\beta_1) + \alpha_\parallel'(2\cos^2\beta_1 \cos^2\beta_2 + 4\sin^2\beta_1 \sin^2\beta_2 + 2\cos^2\beta_2 \sin^2\beta_1 + \cos^2\beta_1 \sin^2\beta_2 + \cos^2\beta_1)]$$

By similar computation it is obtained that

$$n_{3,z}^2 = 1 + \pi[\alpha_\perp'(2\cos^2\beta_1 \cos^2\beta_2 + 4\sin^2\beta_1 \sin^2\beta_2) + \alpha_\parallel'(2\cos^2\beta_1 \sin^2\beta_2 + 2\cos^2\beta_1 + 4\cos^2\beta_2 \sin^2\beta_1)]$$

The difference of the squares of the index of refraction gives

$$(n_{3,z}^2 - n_{3,x}^2) = \pi[(\alpha_\perp' - \alpha_\parallel')(1 - 3\sin^2\beta_1)(1 - 3\sin^2\beta_2)]$$

Therefore

$$\frac{n_{3,z}^2 - n_{3,x}^2}{n_{\mathrm{DNA},z}^2 - n_{\mathrm{DNA},x}^2} = \frac{\Gamma_3}{\Gamma_{\mathrm{DNA}}} = \tfrac{1}{4}(1 - 3\sin^2\beta_1)(1 - 3\sin^2\beta_2)$$

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