$$S(\kappa,\omega) \simeq B\int_0^\infty (1/\kappa^2CM^{-\alpha})(1-\omega/2\kappa^2CM^{-\alpha}) \quad Mf(M)dM \qquad \text{(A-3)}$$

Here the substitution $D = CM^{-\alpha}$ has been made, and B is a group of constants.

The maximum spectral amplitude at zero frequency is

$$S(\omega=0) = (B/2\kappa^2C)\int_0^\infty M^{1+\alpha}f(M)dM \qquad \text{(A-4)}$$

The frequency $\omega_{1/2}$ at which the composite spectrum attains this half-maximum value is found by setting $S(\kappa,\omega)$ as given by eq A-3 equal to one-half the maximum value given by eq A-4 and solving for ω. The result is

$$\omega_{1/2} = \kappa^2C\left[\frac{\int_0^\infty M^{1+\alpha}f(M)dM}{\int_0^\infty M^{1+2\alpha}f(M)dM}\right] \qquad \text{(A-5)}$$

By analogy with the procedure used in defining the viscosity-average molecular weight, we may define a "Rayleigh line-width average" molecular weight by

$$M_c = \left(\frac{\int_0^\infty M^{1+2\alpha}f(M)dM}{\int_0^\infty M^{1+\alpha}f(M)dM}\right)^{1/\alpha} \qquad \text{(A-6)}$$

Then

$$\omega_{1/2} = \kappa^2CM_c^{-\alpha} \simeq \kappa^2D_c \qquad \text{(A-7)}$$

The average M_c defined by eq A-6 does not correspond exactly to any conventional average. However, just as numerical calculation using common distribution functions shows the viscosity-average molecular weight to be close to the weight average, here similar calculations show that M_c is often close to M_z, the z-average molecular weight. (This may be readily verified using a distribution consisting of equal weights of two polymers which differ in molecular weight.)

The circumstances under which the approximations made in deriving eq A-6 will fail to be valid can be determined by numerical calculation. Examination of Tables I and III shows that because D_c/D_z does not deviate greatly from unity in a variety of circumstances, the approximation that in heterodyning experiments $M_c \simeq M_z$ is better than would be expected. Apparently the errors introduced by approximations compensate one another to some extent when an exact numerical calculation including $P(x)$ is made.[16]

(16) We are indebted to the referee for pointing out to us that an alternative means of considering the average obtained which leads to the z-average has been given by P. N. Pusey *et al.* (*J. Phys. (Paris) Colloq., Suppl. 2-3,* **33,** 163 (1972).

Electric Dichroism Studies of Macromolecules in Solutions. II. Measurements of Linear Dichroism and Birefringence of Deoxyribonucleic Acid in Orienting Electric Fields

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ABSTRACT: A method and apparatus for measuring both optical dichroism and birefringence are described. The method is applied to two systems of biological interest, DNA and a DNA-10-Methylacridine Orange complex. Using native calf thymus DNA, these systems are observed experimentally to follow an almost linear rather than quadratic dependence on field strength at low field strengths, as would be predicted for rigid macromolecules with the dielectric properties of DNA if the orienting mechanism is restricted to the torque exerted by the applied electric field on the permanent and induced moments. Since, at the fields used in these studies, saturation of the orientation was not observed, the precise angle which the planes of the base pairs and of the 10-Methylacridine Orange moieties make with the helical symmetry axis of DNA has not been determined; the data show that the 10-Methylacridine Orange planes are approximately parallel to the planes of the bases and both make an angle larger than 60° with the helix axis. The wavelength dependence of the dichroism of DNA reveals the presence of more than one electronic transition moment with different orientations in the region between 225 and 300 nm. The effect of dichroism on birefringence measurements has been analyzed and applied to the observed birefringence of DNA in the 260-nm band.

Precise information on the intramolecular structure of localized or extended segments of such biologically important molecules, such as proteins, nucleic acids, and their synthetic analogs, has been sought by indirect or derivative methods such as chiral activity, nmr, and a host of others, and by direct methods such as X-ray diffraction. The latter, though a powerful tool for unraveling the structure of biological macromolecules which can be crystallized or drawn into fibers, is of little assistance for studying the solution properties. However, these molecules, which frequently incorporate chromophoric groups

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that can act as a structural label, tend to orient in a selective direction, forming uniaxial "quasicrystals," when subjected to external force. The system then absorbs and refracts light polarized parallel or perpendicular to the orienting force with a preference that depends on the anisotropic properties of the molecules. This gives rise to a dichroic spectrum in the wavelength region of the absorption band if the chromophore has a nearly rigid spatial arrangement relative to the molecular axis. Outside the absorption band the linear birefringence dispersion associated with it is normal;[1a] however, it becomes anomalous[1b] near and inside such an absorption band,[2] just as the circular birefringence dispersion (*i.e.*, optical rotatory dispersion) becomes anomalous inside an optically active absorption band (Cotton effect). Once the average degree of orientation of the molecules with respect to the external force field is known, the dichroism or anomalous birefringence gives the orientation of the chromophore and thus directly elucidates intramolecular structure or conformation. The physical property to be ascertained is the angle (or angles) between the transition moment(s) of the chromophore and the principal axis (or axes) of the molecule as a whole.

Current methods employ either flow or electric field to orient molecules in solutions.[3-6] The observed phenomena are accordingly termed flow linear dichroism (and birefringence) and electric linear dichroism (and birefringence). The latter appears superior with regard to the interpretability of the results and experimental versatility. One important drawback of the electric methods is the difficulty of maintaining a sufficiently high electric field in ionic solutions because of electric conduction through the medium. This obstacle may be circumvented partially by pulsing the external electric field.[1,2,4] This technique, moreover, provides a rich dividend of information on the hydrodynamic properties of the oriented molecule from which the overall molecular dimensions may be deduced.

Electric dichroism and birefringence, both of which are signed effects, can in principle be observed for any kind of a molecule with conformationally fixed chromophores, since these effects result from interaction between the external electric field, and the transition moment, the ground state electric permanent dipole moment, and the anisotropic polarizability. When the strength of the field is exceedingly high, additional effects due to the interaction of the field with the excited electronic state as well as the transition moment itself should become appreciable. Such effects may result in a shift of the absorption band (change in energy) or alteration of the band shape (change in transition probability) as discussed in a preceding paper (Part I).[7]

Electric birefringence is in general a more sensitive index than dichroism and may be the only feasible one if the entire absorption band is not easily accessible.[8] These facts make electric birefringence the method of choice for determining the electrooptical properties of simple polypeptides,[8] smaller molecules,[9,10] and globular polymers[11] which are more difficult to orient because of their usually smaller permanent or inducible dipole moments. Using the Kronig–Kramers-type transform for data conversion, birefringence measured through an absorption band yields the same structural information as does dichroism.[12] However, determination of birefringence or optical phase retardation from a measurement of the intensity of light transmitted through crossed polarizers is more subject to artifacts from stray birefringence and other undesirable polarization phenomena.[8,13] These become particularly apparent inside an absorption band where the observed birefringence is a complicated sum of true birefringence and the effect of dichroic absorption, as will be demonstrated in this paper. Unless the latter effect is removed, the conclusions drawn are often misleading.

In this paper we report methods for measuring both electric dichroism and birefringence inside an absorption band, describe an apparatus which utilizes the pulse technique and present some data on the dichroism of native deoxyribonucleic acid (DNA) and a DNA-dye complex as functions of both wavelength and electric field strength. We discuss these observations by comparing them with theoretical calculations in a preceding paper.[7] Finally, we present a comparative study of electric dichroism and anomalous birefringence dispersion through the 260-nm absorption band of a native DNA in 10^{-3} N NaCl solution in order to show to what extent the dichroic absorption distorts the shape of the true dispersion and in what manner the data may be corrected to account for this.

Apparatus

The design of the apparatus is predicated on the desire to: (1) measure both electric dichroism and birefringence with minimum handling of the optical system, (2) include a wide spectral range in the visible and ultraviolet spectrum, and (3) utilize electric field strengths up to 33 kV/cm or more, which can be applied in the form of a single square pulse for as long as a few milliseconds. The apparatus consists of three major parts (optical, electrical, and measuring cell), each of which will briefly be described below.

Optical Components (Figure 1). *Monochromator.* The monochromator of a Beckman DU spectrophotometer (Model 2400) is used together with its lamp house. The monochromator is calibrated to better than 0.1 nm with a Beckman No. 2260 mercury lamp.

Light Source. The Beckman 96280 deuterium lamp, which proved to provide the maximum stable intensity at 250 nm, is employed below 340 nm, above which a tungsten lamp is used as the light source. These lamps are all operated with the dc power source to avoid modulation problems with the single wave pulse method. The electric power is supplied with a 6-V storage battery for the tungsten lamp and through a Model B Beckman hydrogen power supply for the deuterium lamp.

Optics. Two double, convex quartz lenses of Suprasil grade, both of which have a focal length of approximately 3.5 cm, are used at positions L_1 and L_2. Lens L_1 focuses the slightly diverging beam from the exit slit of the monochromator at the center of a "Kerr' cell and lens L_2, in turn, focuses the image on a photomultiplier detector. This arrangement is necessary to obtain the maximum transmitted light intensity falling on the detector and to make the cross-section of the incident beam small enough to avoid striking the parallel plate electrodes, the smallest gap of

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(1) (a) J. R. Partington, "An Advanced Treatise on Physical Chemistry," Vol. 4, Longmans, Green and Co., London, 1953, p 278. (b) Anomalous is used here in the customary sense that the curve of birefringence *vs.* wavelength is not monotonic but instead has inflection points.

(2) S. H. Lin, C. Y. Lin, and H. Eyring, *J. Phys. Chem.*, **70**, 1756 (1966).

(3) C. T. O'Konski, "Encyclopedia of Polymer Science and Technology," Vol. 9, Wiley, New York, N. Y., 1968, p 551.

(4) K. Yoshioka and H. Watanabe in "Physical Principles and Techniques of Protein Chemistry," Part A, S. Leach, Ed., Academic Press, New York, N. Y., 1969, p 335.

(5) R. E. Harrington, "Encyclopedia of Polymer Science and Technology," Vol. 7, Wiley, New York, N. Y., 1967, p 100.

(6) E. Charney, *Procedures Nucl. Acid Res.*, **2**, 176 (1971).

(7) K. Yamaoka and E. Charney, *J. Amer. Chem. Soc.*, in press.

(8) K. Yamaoka, Ph.D. Thesis, University of California, Berkeley, 1964.

(9) W. H. Orttung and J. A. Meyers, *J. Phys. Chem.*, **67**, 1905, 1911 (1963).

(10) E. Charney and R. S. Halford, *J. Chem. Phys.*, **29**, 221 (1958).

(11) W. H. Orttung, *J. Amer. Chem. Soc.*, **87**, 924 (1965).

(12) A. Moscowitz *in* "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill, New York, N. Y., 1960, p 150.

(13) (a) E. Charney, *J. Opt. Soc. Amer.*, **45**, 980 (1955); (b) *ibid.*, **51**, 912 (1961).

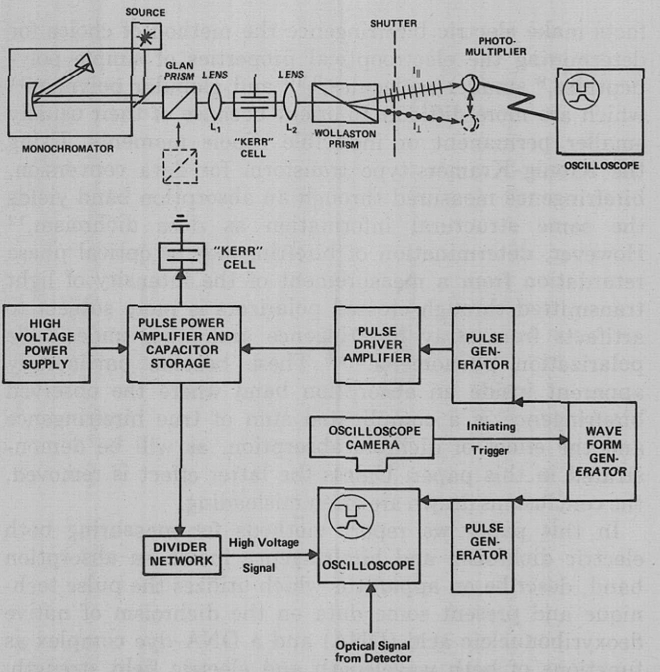

Figure 1. Schematic diagram of optical component of apparatus for electric dichroism and birefringence measurements (A, top); block diagram of apparatus (B, bottom).

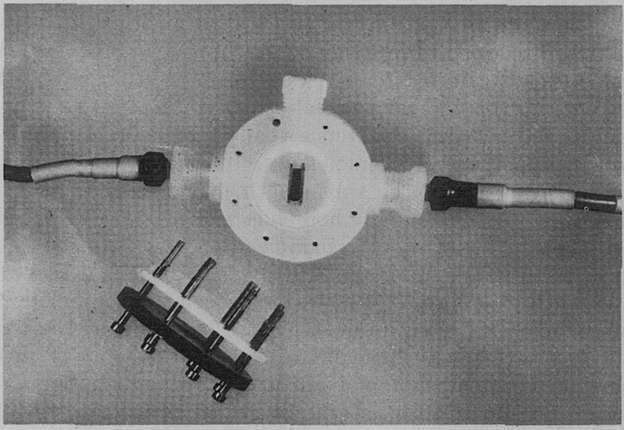

Figure 2. Photograph of "Kerr" cell. For details, see text.

which is about 0.12 cm. Since lens L_1 is achromatic, both its position and that of the detector are adjusted between the visible and ultraviolet spectrum. A Glan-type polarizer made of calcite, the rotation angle of which can be read with an accuracy of 0.001°, is mounted on a rotatable, backlashless mount made by Mr. Charles Lohr of this laboratory. The mount is removed from the optical bench for electric dichroism measurements. A beam-splitting Wollaston-type analyzer, made of natural quartz with a divergence of about 3° between the perpendicularly polarized rays, is placed between the lens L_2 and the detector on a mount rotatable about its axis with an accuracy of 0.1°. The separation of the two beams is about 1 cm at the position of the detector approximately 10 cm from the analyzer. Both polarized rays are utilized for the measurement.

Detector. The transmitted polarized light intensity is detected with an RCA 1P21 photomultiplier above, or a 1P28 below, 320 nm. The photomultiplier is housed in an antimagnetic metal tube to avoid magnetic pickup. The housing can be moved laterally with a shaft from outside the dust- and light-proof enclosure which accommodates all optical elements so that the parallel and perpendicular rays can be detected individually. Whenever needed, dry nitrogen gas is flushed through the enclosure to prevent the cold cell from dewing. The photomultiplier tubes are operated between 500 and 1000 V, supplied from a Keithley Model 246 high-voltage power supply.

The "Kerr" Cells (Figure 2). The quartz cuvet cells, which have often been used in electric birefringence measurements,[3,8,14] are usually not completely free from residual birefringence that introduces an error (see Methods). In the present work a "Kerr" cell of different type was designed. This cell, fabricated from a Kel-F rod, has an H-shaped opening in the center. The arms of the H are fitted with highly polished stainless steel or gold-plated brass electrodes with a fixed height of 1 cm and appropriate length. The sample solution occupies the central portion of the H; the amount required ranges between 0.5 and 1.5 ml depending on the distance between the parallel plate electrodes. A Teflon O ring is embedded in the groove on each face of the Kel-F block and skimmed off in a lathe to ensure flatness. Strain-free quartz window disks are gently pressed on the O rings from both ends *via* polyethylene pad and brass guard plate. Electric contact with the parallel plate electrodes is made through pressure-tight brass pins from both side arms of the block. To achieve a good ohmic contact, a small piece of pure indium metal is placed between the pin and the electrode. Two Kel-F side arms are machined to be exactly perpendicular to the parallel electrodes. The cell is precisely and reproducibly positioned in the optical path inside the two halves of a blackened hollow brass thermostatable block through which thermoregulated liquid is circulated to maintain the sample at constant temperature. The sample solution is injected through the larger of two openings on the top of the cell with a hypodermic syringe or glass pipet; the smaller hole acts as an air escape vent. A pinhole is drilled in the top screw cap so that the pressure inside the cell remains atmospheric.

Electric Components. *Pulse Delivering System.* Electric components for delivering a single square wave electric pulse of varying duration and voltage consist of four major parts: pulse generator unit, pulse amplifier unit, dc high-voltage supply unit, and pulse discharge network. The pulse generator unit is an assembly of Tektronix, type 160A power supply, type 162 waveform generator, and two type 161 pulse generators. The function of each unit has been described in detail elsewhere[8] and will not be repeated here. The rest of the electronic units were constructed in this laboratory and are very similar to but not identical with the previous ones;[8] the circuit diagrams are available from the authors. The dc high-voltage supply delivers up to 4 kV continuously providing electric fields of 33 kV/cm with a 0.12-cm wide "Kerr" cell. The driver amplifier can produce a single square wave pulse up to 5-msec long duration with negligible distortion unless the solution conductivity is greater than that of 10^{-3} *M* NaCl. The pulse discharge network is essentially the same as the one described in ref 8. The voltage of the pulse generated across the "Kerr" cell is measured through a precision divider network consisting of a series of carbon resistors and a Tektronix P6008 attenuator probe. The total reduction ratio is 93:1 in the present apparatus. This reduced pulse is fed into one channel of a 1A1 plug-in preamplifier of a 535A Tektronix oscilloscope.

Signal Detecting System. The output voltage generated across an anode resistor by the photomultiplier current is fed to the other channel of the oscilloscope preamplifier *via* a capacitor network which constitutes a filter unit in combination with the anode resistor circuit when a steady-state dichroism signal with improved signal to noise ratio is desired; however, this is done at the expense of distortion in the transient signal. To obtain an undistorted transient signal such as a rapid rise and decay process, the capacitor must be bypassed and the lowest possible anode resistor is used to minimize the resistor circuit time constant. The oscilloscope is operated at chopped mode to display simultaneously both pulse and photosignal on the screen. These signals are recorded with a C-12 Polaroid camera attachment in combination with a projected graticule. All signals including the stationary dc light levels are photographed on a single film. The oscilloscope amplitude calibrator is calibrated with an external standard voltage generator and used as the secondary standard for the calibration of the gain of the 1A1 plug-in unit in day-to-day operation. The ac line voltage is regulated at exactly 120 V controlled with a Sorensen Model 2000S ac voltage regulator. All electrical units are provided with a common ground and care is taken to avoid ground loops.

Methods

The well-collimated monochromatic light emerging from a monochromator is, in general, partially polarized depending on

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(14) C. T. O'Konski and A. J. Haltner, *J. Amer. Chem. Soc.*, 78, 3604 (1956).

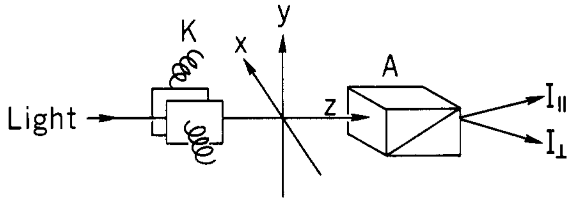

Figure 3. Schematic of electric dichroism.

the characteristics of the monochromator. The total incident light intensity, I, is then considered to consist of both natural and polarized components, I^{p} and I^{u}, the latter of which may be proportional to I with proportionality constant k.

$$I = I^{\mathrm{u}} + I^{\mathrm{p}}$$
$$I^{\mathrm{u}} = kI \qquad (1)$$
$$I^{\mathrm{p}} = (1 - k)I$$

Electric Dichroism[15a] **(Figure 3).** The intensity of the light beams emerging from the appropriately oriented Wollaston prism may be derived as follows. Let the intensities of the emerging beam polarized parallel to the direction of external electric field be $I_{\ ,\mathrm{E}}$ and $I_{\ ,0}$, respectively, in the presence and absence of the field. The relations between these two quantities are given by

$$I_{\parallel,\mathrm{E}}/I_{\parallel,0} = 10^{-(\epsilon_{\parallel}-\epsilon_0)cl} = 10^{-\Delta A_{\parallel}} \qquad (2)$$

$$-\Delta A \ = \log\,[1 + (\Delta I\ /I_{\ ,0})] \qquad (3)$$

where $\epsilon_{\parallel}$ and ϵ_0 are the molar extinction coefficients of a chromophore in the presence and absence of the external field; c is the concentration in mol/l.; l is the optical path length of the "Kerr" cell (the correction of the end effect is described elsewhere[15b]); $\Delta A\ = A\ - A_0$[15c] is the difference in absorbances; and $\Delta I\ = I_{\parallel,\mathrm{E}} - I_{\parallel,0}$ is a quantity determined by measuring the oscilloscope display of the dichroic photosignal.

Correspondingly, for the emerging beam polarized perpendicular to the external field with total transmitted intensities, $I_{\perp,\mathrm{E}}$ and $I_{\perp,0}$, in the presence and absence of the field

$$-\Delta A_{\perp} = \log\,[1 + (\Delta I_{\perp}/I_{\perp,0})] \qquad (4)$$

Now the reduced dichroism may be calculated from the individual dichroism measurements as shown in eq 5. It can be proved in a straightforward manner that it is immaterial whether the light incident on the "Kerr" cell is partially polarized or not when the dichroism measurement is made with substantially perfect polarizers.[13]

$$\frac{\Delta\epsilon}{\epsilon_0} = \frac{\epsilon_{\parallel} - \epsilon_{\perp}}{\epsilon_0} = \frac{\Delta A_{\parallel} - A_{\perp}}{A_0} = \frac{1}{A_0} \times \log\left(\frac{1 + (\Delta I_{\perp}/I_{\perp,0})}{1 + (\Delta I_{\parallel}/I_{\parallel,0})}\right) \qquad (5)$$

Electric Birefringence (Figure 4). Either the polarizer or analyzer may be set at a fixed angle and the other rotated. With an absorbing sample in the "Kerr" cell, the intensity of polarized light emerging from the analyzer, I_{an}, is given by[8] eq 6, where I^{p} is the intensity of the polarized light incident on the sample cell; α and β are the angles between the plane of polarization and the direction of the external field, respectively, for polarizer and for analyzer; δ is the optical phase retardation between the x and y axes. W and γ are related to the absorption of polarized light by the sample

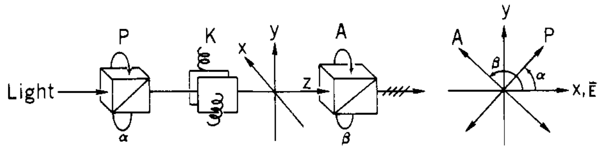

Figure 4. Schematic of electric birefringence.

$$I_{\mathrm{an}}/I^{\mathrm{p}} = (W/4)\{1 + \cos 2\alpha \cos 2\gamma + (\cos 2\gamma + \cos 2\alpha) \cos 2\beta + \sin 2\alpha \sin 2\gamma \sin 2\beta \cos \delta\} \qquad (6)$$

$$W = e^{-\kappa_x l} + e^{-\kappa_y l} \qquad (7)$$

$$\tan\,\gamma = e^{(l/2)(\kappa_x - \kappa_y)} \qquad (8)$$

where κ_x and κ_y are absorption coefficients along the x and y axes and are in general equal to each other in the absence of the orienting field.

For completeness and to demonstrate the method utilized in our laboratory, we write the expressions which are applicable in the presence of imperfections in the polarizers or residual birefringence, δ^0, of the "Kerr" cell. With the polarizers crossed and individually passing light vectors at 45° to the external field direction (using eq 1, 6, and 8, $\alpha = \pi/4$ and $\beta = 3\pi/4$) the light transmitted through the analyzer, $\Delta I_{\mathrm{an}}{}^0$, is given in terms of quantities which are directly proportional to the experimentally observed voltages

$$\Delta I_{\mathrm{an}}{}^0/I^{\mathrm{p}} = (I_{\mathrm{an}}{}^0 - I_{\mathrm{d}})/I^{\mathrm{p}} = (W^0/4)(1/(1 - k) - \cos \delta^0) \qquad (9)$$

where I_{d} is the dark current of the photomultiplier. If, still in the absence of the field, the polarizer angle is offset from $\pi/4$ by the angle $\Delta\alpha$, then

$$\Delta I_{\mathrm{an}}{}^{0\prime}/I^{\mathrm{p}} = (W^0/4) \cos \delta^{\circ} \sin^2 \Delta\alpha \qquad (10)$$

With the polarizer reset to $\alpha = \pi/4$, the application of the external field makes the sample in the "Kerr" cell birefringent and produces a phase difference, δ^{E}, and an extinction difference measured by γ^{E} between the polarized components of the radiation parallel, x, and perpendicular, y, to the external field direction. The difference between the intensities transmitted by the system under these conditions and under the same conditions in the absence of the field is as shown in eq 11. To eliminate $1/(1 - k)$, eq 9, 10, and 11 are combined to give eq 12, where referring to eq 3 and 4, eq 13–15 are derived. Equations 12–15 clearly indicate that *both* parallel and perpendicular dichroism (not only the difference) are required to calculate the retardation, $\Delta\delta$, which is proportional to birefringence, whenever the effect of dichroism as well as monochromism must be taken into account.

$$\Delta I^{\mathrm{E}}/I^{\mathrm{p}} = I^{\mathrm{E}}/I^{\mathrm{p}} - \Delta I_{\mathrm{an}}{}^0/I^{\mathrm{p}} = (W^0/4)\{((W^{\mathrm{E}}/W^0) - 1) \times [1/(1 - k)] - (W^{\mathrm{E}}/W^0) \sin 2\gamma^{\mathrm{E}} \cos \gamma^{\mathrm{E}} + \cos \delta^0\} \qquad (11)$$

$$\left(\frac{\Delta I^{\mathrm{E}} + \Delta I_{\mathrm{an}}{}^0 - (W^{\mathrm{E}}/W^0)\Delta I_{\mathrm{an}}{}^0}{\Delta I_{\mathrm{an}}{}^{0\prime}}\right) \sin^2 \Delta\alpha = \frac{1}{2} \times \frac{W^{\mathrm{E}}}{W^0}\left(1 - \frac{\cos \delta^{\mathrm{E}}}{\cos \delta^0} \times \sin 2\gamma^{\mathrm{E}}\right) \qquad (12)$$

$$\frac{W^{\mathrm{E}}}{W^0} = \frac{1}{2}\left(10^{-\Delta A_{\parallel}} + 10^{-\Delta A_{\perp}}\right) = \frac{1}{2}\left\{\left(1 + \frac{\Delta I_{\parallel}}{I_{\parallel,0}}\right) + \left(1 + \frac{\Delta I_{\perp}}{I_{\perp,0}}\right)\right\} \qquad (13)$$

$$\sin 2\gamma^{\mathrm{E}} = \frac{2 \times 10^{\Delta A/2}}{1 + 10^{\Delta A}} = \frac{2(1 + \Delta I_{\parallel}/I_{\parallel,0})^{1/2}(1 + \Delta I_{\perp}/I_{\perp,0})^{1/2}}{(1 + \Delta I_{\parallel}/I_{\parallel,0}) + (1 + \Delta I_{\perp}/I_{\perp,0})} \qquad (14)$$

$$\delta^{\mathrm{E}} = \delta_x{}^{\mathrm{E}} - \delta_y{}^{\mathrm{E}} + \delta^0 = \delta\ {}^{\mathrm{E}} - \delta\ {}^{\mathrm{E}} + \delta^0 = \Delta\delta + \delta^0,\ \Delta A = \Delta A\ - \Delta A \qquad (15)$$

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(15) (a) Throughout this work we assume that either the sample is optically inactive or the effect of optical activity on the observed electric linear dichroism or birefringence is negligibly small; also that ordinary "Rayleigh" scattering is sufficiently small that anisotropy of scattering produced by the electric field does not contribute to the difference in the intensities of the transmitted light signals in the presence and absence of the applied electric field. (b) L. Chaumont, *Ann. Phys.*, **5**, 31 (1916). (c) Isotropic absorbance is designated by A^0 or A_0 from which no confusion should arise. In general both subscript and superscript zero are used to indicate the value of a quantity measured in the absence of an applied electric field.

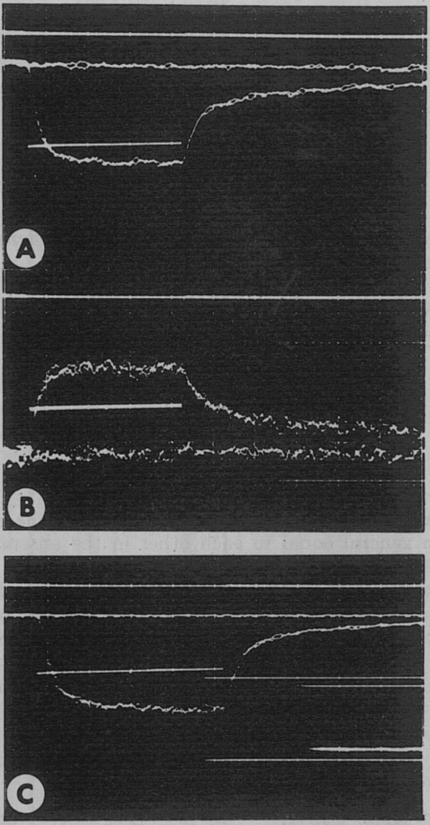

Figure 5. Photographs of electric dichroism and birefringence of a DNA sample in 10^{-3} *N* NaCl solution. Time scale: 0.02 msec/division.

If the effect of monochromism is negligible, then eq 13 reduces to

$$W^{E}/W^{0} = (1/2)(10^{-2\Delta A/3} + 10^{\Delta A/3}) \tag{13a}$$

since $A^{E} = A_0 = (A_{\parallel} + 2A_{\perp})/3$.

If the dichroism effect is ignored, eq 12 reduces to

$$\frac{\Delta I^{E}}{\Delta I_{an}{}^{0\prime}} = \frac{\sin\left(\left(\dfrac{\Delta\delta}{2} + \delta^{\circ}\right) \times \sin\left(\dfrac{\Delta\delta}{2}\right)\right)}{\sin^2 \Delta\alpha \times \cos \delta^{\circ}} \tag{16}$$

since $\sin 2\gamma^{E}$ and W^{E}/W^{0} should both be unity.

It is evident from eq 12 and 16 that unless the residual birefringence is known, the optical retardation due to the applied field cannot be calculated. Since δ^0 is very difficult to evaluate, it should always be minimized. In practice, this is accomplished by choosing strain-free window plates and by exercising reasonable care in tightening them against the cell block. (A variable optical retarder may, of course, be placed in the beam after the "Kerr" cell and set to cancel the residual birefringence of the cell, but this has the disadvantage of introducing still another optical element in the beam.) In this case eq 12 and 16 may be solved for $\Delta\delta$ by equating $\cos \delta^0 = 1$. In summary, the following three quantities are recorded on the oscilloscope screen for electric birefringence measurements: ΔI^{E} (birefringence signal), $\Delta I_{an}{}^{0\prime} = (I_{an}{}^{0\prime} - I_{an}{}^{0} + I_d)$ and $\Delta I_{an}{}^{0} = (I_{an}{}^{0} - I_d)$.

Electric Dichroism with Birefringence Apparatus. Before closing this section, we note that the electric dichroism measurement can be carried out with a conventional electric birefringence apparatus which employs a Rochon type polarizing prism as analyzer. There are two methods for such a measurement. (a) The angle of the polarizer is fixed at 45°, exactly the same as for birefringence measurements and the analyzer only is rotated to either $\beta = 0°$ or $\beta = 90°$. (b) Both analyzer and polarizer are rotated together so that either $\alpha = 0$ and $\beta = 0$ or $\alpha = 90°$ and $\beta = 90°$. In this latter method the signals are twice as strong as the former and precisely equal to those obtained with the dichroism apparatus described in this work, since the two prisms are set at the same polarization angle. Unless the optical material of the prism absorbs light, this method may be superior to the presently described one in that adjustment of the detector position caused by the removal of the prism from the optical path is unnecessary and that both dichroism and birefringence can be measured alternately.

Measurements

The methods of measurement described here all presuppose that (1) the Malus law of cosines is valid for the optical setup and (2) the output signal voltage displaced on the oscilloscope is linearly proportional to the number of photons incident on the photomultiplier tube. These were tested and found quite satisfactory.

The procedure followed for electric dichroism measurements consists of measuring the static light intensity transmitted through the sample cell and other optics, say $I_{\parallel,0}$, by the difference between the two dc output signals as recorded on an oscilloscope photograph when the beam is blocked by the shutter and when it is not. Then the input channel for the photosignal is switched to ac response, the electric field triggered and the resulting dichroism signal photographed (*cf.* Figure 5A,B). The magnitude of this signal $\Delta I_{\parallel}$ is given as the distance between the base line and the steady-state signal level, multiplied by the scope amplifier gain factor used in the measurement.

For electric birefringence measurements, the light intensity transmitted through the crossed polarizer prisms, $I_{an}{}^{0}$, is first recorded on the oscilloscope photograph (Figure 5C) followed by the photomultiplier dark current, I_d. These two dc signal levels occupy about a third of the photographic picture (on the right in Figure 5C). Thus $\Delta I_{an}{}^{0}$ can be determined. Another static light intensity, $\Delta I_{an}{}^{0\prime}$, transmitted through the prisms, with the analyzer offset by $\Delta\alpha$ as described above, is determined from the distance between the dc signal level and the signal level at $\Delta\alpha = 0$, both extending horizontally across about a half of the same photograph. After the analyzer is rotated back to the cross position, the transient signals, base line, and electric field induced optical retardation are recorded, again using ac coupling. The magnitude of the retardation is determined as the distance between the two signal levels; all signals multiplied by the scope amplifier gain factor used in the particular measurement.

In both dichroism and birefringence measurements, the spectral resolution is kept constant by setting the slit width manually at each wavelength to a desired value. In the measurements reported here a half-intensity band width of 25 Å was utilized. In conducting solutions, the applied field initially reaches a maximum and then steadily decreases; the signal was always read at the temporal midpoint of the square pulse. Native DNA in 10^{-3} *N* NaCl solution is stable to the electric pulse for at least three or four repetitive applications at about 30-sec intervals unless the applied field is exceedingly high.

Results

Electric Dichroism of DNA in the Ultraviolet Spectrum. *Wavelength Dependence.* As a typical example of the uv electric linear dichroism, the parallel dichroism $(A_{\parallel} - A_0)$, the perpendicular dichroism $(A_{\perp} - A_0)$ and the dichroism $(A_{\parallel} - A_{\perp})$ of native calf thymus DNA in 10^{-3} *N* NaCl solution are shown in Figure 6. The maximum and minimum isotropic absorbance of the solution were 1.57 at 258 nm and 0.19 at 295 nm, respectively. A hyperchromic value of 35% at 259 nm upon thermal denaturation was taken as a criterion of the native state of the sample.[16]

From these data, the reduced dichroism was calculated and is shown in Figure 7 together with an isotropic spectrum of the same native DNA solution which was taken with a Cary Model 14 spectrophotometer at room temperature. The reduced dichroism is essentially constant between 245 and 277.5 nm where the isotropic spectrum shows an apparent absorption maximum at 258.5 nm. At the longer wavelengths, the reduced dichroism decreases with two apparent inflection points. To ascertain whether or not the long-wavelength dichroism reverses its sign, a tenfold-concentrated DNA solution was used to extend the

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[16] K. Yamaoka and R. A. Resnik, *J. Phys. Chem.*, **70**, 4051 (1966).

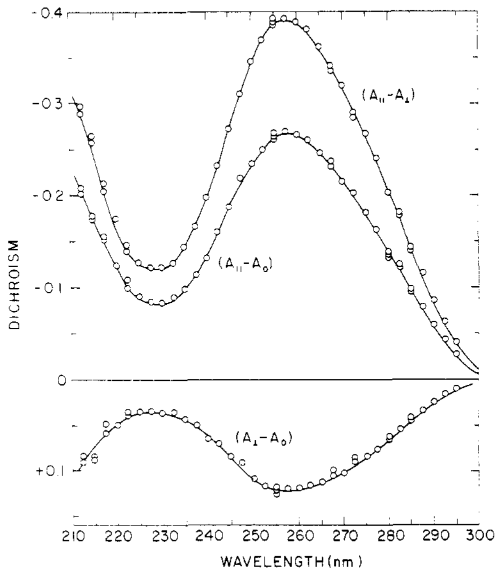

Figure 6. Electric dichroism spectra of native calf thymus DNA in 10^{-3} *N* NaCl solution at a constant electric field strength of 4750 V/cm, pH 6.4, 25°. The concentration of DNA was 2.43×10^{-4} *M* as determined photometrically (*cf.* ref 16). The stock solution (4 mg/ml) was dialyzed against distilled water containing 10^{-4} *N* NaCl, and then diluted with aqueous 10^{-3} *N* NaCl solution. The hyperchromicity determined as cited in ref 16 was 35%. The DNA sample was purchased from Worthington Biochemical Corp., Freehold, N. J. The ordinate is the parallel, perpendicular, or reduced dichroism expressed in terms of the difference in absorbances calculated from eq 3, 4, and 5. Circles are measured points. A 1-cm long, 0.2-cm wide "Kerr cell" was used throughout this work.

dichroism measurement (as shown with open triangles in Figure 7). No reversal was observed; however, there is a concentration dependence of dichroism such that at fixed field strength an increase in concentration decreases the magnitude of the dichroism, which is perhaps indicative of a conformational change, ion binding, or intermolecular aggregation. At wavelengths shorter than 245 nm, the dichroism values also decrease gradually but reach a minimum at about 222.5 nm and then increase, showing no reversal of sign.

Electric Field Strength Dependence. In order to estimate the highest possible dichroism value for the 260-nm band of native calf thymus DNA, the field strength dependence of the parallel and perpendicular dichroism was explored. The results are shown in Figure 8 where the specific dichroism is plotted against the square of the external electric field strength. The magnitudes gradually but steadily increase with increasing field strength; however, no saturation effect is observed up to 8 kV/cm. Nor does a predicted[7] dependence on the square of the applied field at low field strengths appear to hold true. The same dichroism values are then replotted against the first order of the external field strength in Figure 9 to visualize the dependency. It is clearly seen that except at very low fields the field dependence of the dichroism is apparently linear and that the onset of saturation does not appear. The field dependence of the dichroism was measured at two different wavelengths (270 and 245 nm). Interestingly these measured points all fall on the same curves, which suggests that there is no band shift of the 260 nm absorption by electric fields up to 8 kV/cm for a native DNA solution.

Electric Dichroism of a DNA-Dye Complex in the Visible Region. *Wavelength Dependence.* As a typical example of electric dichroism spectrum in the visible region, the wavelength dependence of a complex of native DNA with 10-methyl-3,6-dimethylaminoacridinium chloride (or 10-Methylacridine Orange) in 10^{-3} *N* NaCl solution was studied. The results are shown in Figures 10 and 11 together with the isotropic spectrum for the spectral region between 540 and 410 nm. The maximum and minimum absorbances are 1.62 at 504 nm and 0.024 at 540 nm, respectively, with a 1-cm path-length cell and a fixed concentration. The accuracy of the measurement is sufficient

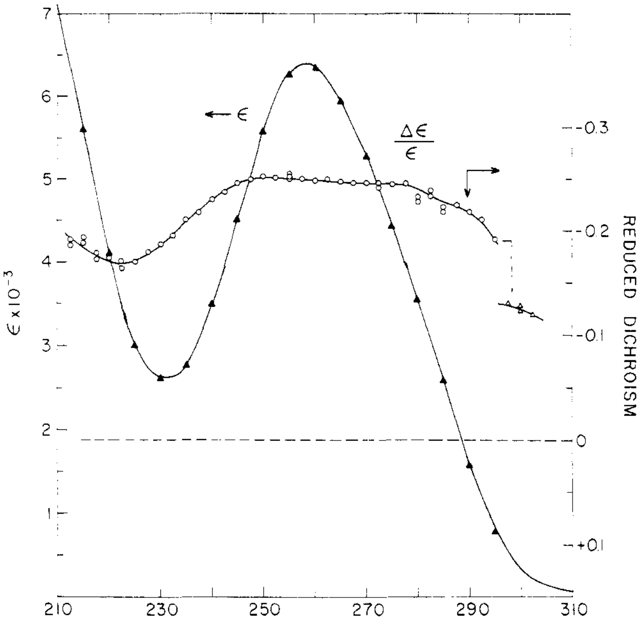

Figure 7. Reduced dichroism spectrum of native DNA together with the isotropic spectrum. Open triangles are for a DNA sample whose concentration is approximately 15 times higher than the sample shown in Figure 6. Other conditions are the same as in Figure 6. The filled triangles are the reconstituted absorbance of the isotropic spectrum as calculated from eq 18. The left ordinate is the isotropic molar extinction coefficient and the right ordinate is the reduced as well as specific dichroisms.

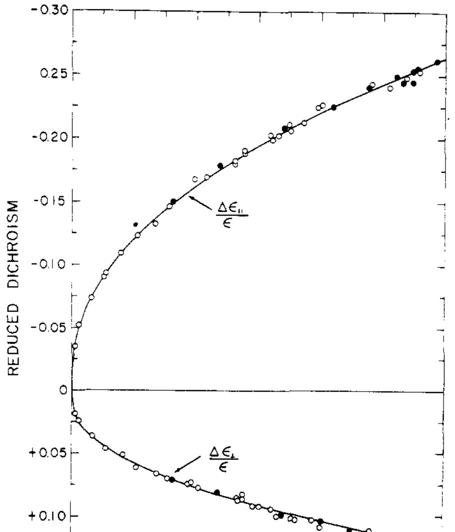

Figure 8. The quadratic electric field strength dependence of the specific parallel and perpendicular dichroism of native DNA in 10^{-3} *N* NaCl. Open circles at 270 nm and filled circles at 245 nm. Solid curves are least-squares fit to the data.

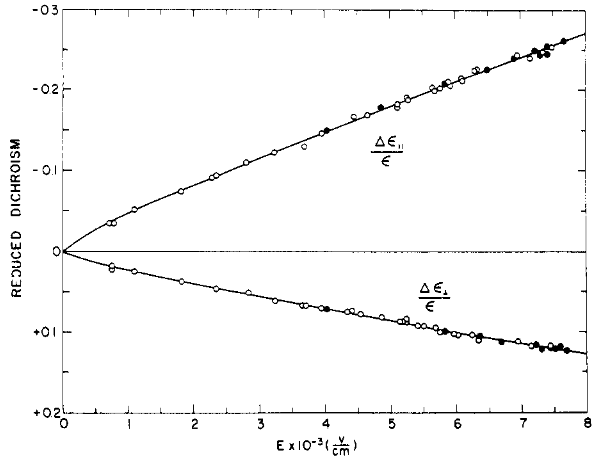

Figure 9. The electric field strength dependence of the specific parallel and perpendicular dichroism of native DNA. Data are the same as shown in Figure 8. Solid curves are least-squares fit to the data.

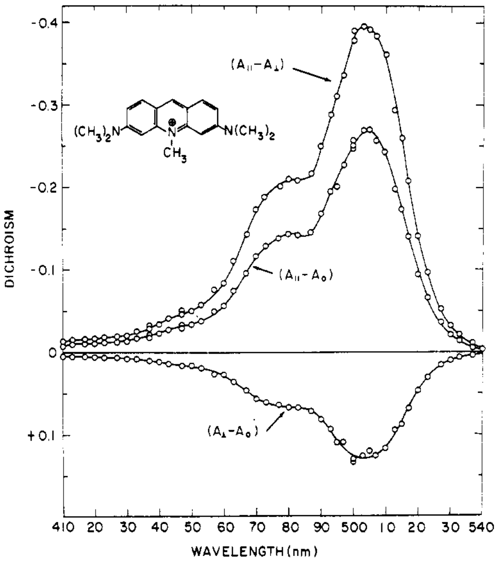

Figure 10. Electric dichroism spectra of 10-methyl-3,6-dimethylaminoacridinium chloride bound to native DNA in 10^{-3} *N* NaCl solution at a constant electric field of 4750 V/cm, pH of 6.4, 25°. The concentration of DNA was 2.43×10^{-4} *M*. The concentration of the dye was 3.40×10^{-5} *M* and the apparent ratio of DNA-P to dye in solution was 8.05. The ordinate is the dichroism expressed in the same manner as in Figure 6. Circles are measured points.

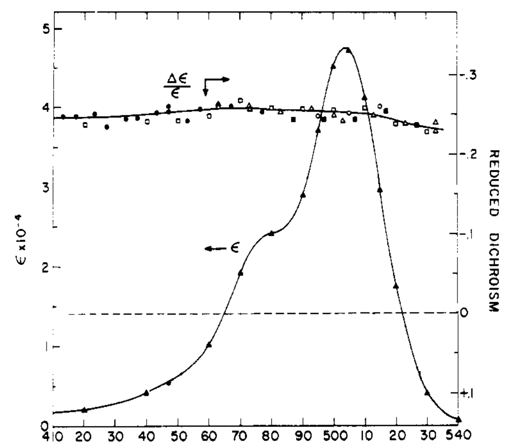

Figure 11. Reduced dichroism spectrum of 10-methyl-3,6-dimethylaminoacridinium chloride in the presence of native DNA in 10^{-3} *N* NaCl together with the isotropic spectrum. Filled triangles are the reconstituted absorbance of the isotropic spectrum as calculated from eq 18. All other symbols are points measured in separate experiments to indicate reproducibility of measurements.

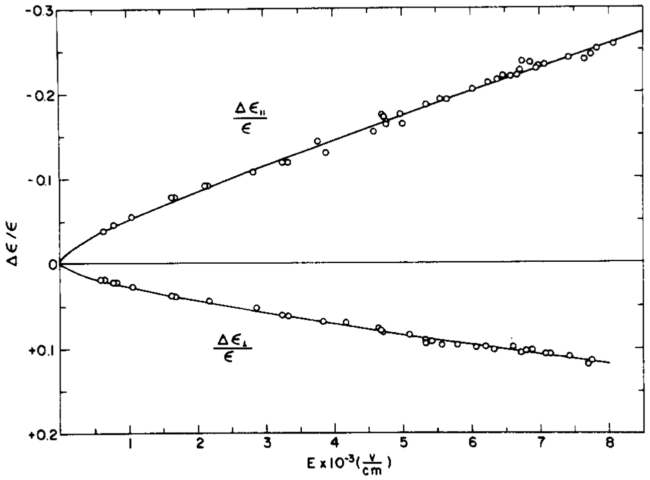

Figure 12. The electric field strength dependence of the specific parallel and perpendicular dichroism of the complex of native DNA formed with 10-methyl-3,6-dimethylaminoacridinium chloride at 480 nm. Solid curves are least-squares fit to the data.

to give reliable dichroism data in the entire region. Although this DNA-dye complex fluoresces moderately, no effect on the absorption was detected. All three dichroism plots in Figure 10 show the same spectral features as those of the isotropic spectrum; that is, a major maximum at 504 nm, a prominent shoulder at about 480 nm, and another barely discernible shoulder at about 440 nm. The sign of the parallel dichroism is negative throughout the band just as for DNA itself. In spite of these features, the reduced dichroism is practically constant inside the absorption band as shown in Figure 11. To compute the data, all dye molecules in solution are assumed to bind to DNA sites.[16] On the longer wavelength side of the isotropic absorption maximum the reduced dichroism decreases somewhat, while a slight increase appears in the 480-nm shoulder region. Otherwise, there is no significant change in magnitude or sign of the dichroism.

Electric Field Dependence. The field strength dependence of the specific parallel and perpendicular dichroism is plotted in Figure 12 against the first order of the applied field strength. Here again, the specific dichroism is, except at low fields, almost linearly proportional to the electric field up to 8 kV/cm and still does not approach saturation. In addition, the curves for the DNA-dye complex are essentially superimposable on the curves for the native DNA shown in Figure 9.

Electric Birefringence inside the 260-nm Absorption Band of Native DNA. *Wavelength Dependence.* The result of the electric birefringence of a native calf thymus DNA solution, which is the same as used for the electric dichroism measurement, is shown in Figure 13. These data were taken under the same optical and electric conditions as used for those of Figure 6. The sign of the observed birefringence is presumed to be negative at 320 nm, since a negative value has been established as a result of measurements in the visible region[17,18] and since in this work the birefringence dispersion was found to be

(17) C. T. O'Konski and N. C. Stellwagen, *Biophys. J.*, 5, 607 (1965).

(18) J. L. Sarquis and R. E. Harrington, *J. Phys. Chem.*, 73, 1685 (1969).

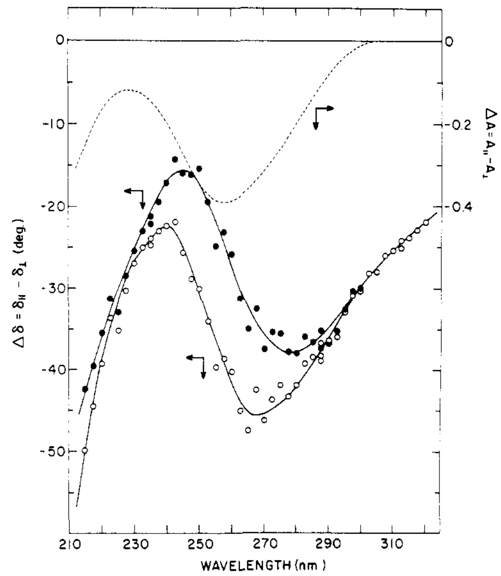

Figure 13. Anomalous electric birefringence dispersion of native calf thymus DNA in 10^{-3} N NaCl solution at a constant electric field of 4750 V/cm, pH 6.4, 25°. The broken curve is the electric dichroism of the same solution for comparison. The electric birefringence is expressed in terms of optical retardation $\Delta\delta$ in degrees. Open circles are points of uncorrected retardation computed from eq 16, whereas filled circles are those which take into account the effect of electric dichroism and are calculated from eq 12, 13, and 14.

monotonically increasing in absolute magnitude down to 320 nm.

To illustrate the contribution of dichroic absorption to birefringence dispersion inside the 260-nm absorption band, the optical retardation, $\Delta\delta$, was first calculated with the aid of eq 16, which assumes no effect of electric dichroism on $\Delta\delta$. C. Houssier and Hans-G. Kuball (*Biopolymers*, **10**, 2421 (1971)) have also recently treated this effect with the same result. These values are represented by open circles in Figure 13. The birefringence dispersion of the DNA solution becomes anomalous as expected and shows a negative trough at 265 nm and a peak at 240 nm, the apparent amplitude (according to the ORD convention) being about 25°. This negative anomalous dispersion effect, analogous to the Cotton effect, is overlapped with another negative dispersion lying at shorter wavelengths. Next, the true optical retardation was calculated with necessary corrections for the effect of electric dichroism on the observed birefringence. The values calculated with the aid of eq 12, 13, and 14 are shown in the same figure with filled circles. It is evident *that electric dichroism has a profound effect on the electric birefringence dispersion inside an absorption band* when an intensity measuring method is employed. The correspondence of birefringence to the observed dichroism now appears to have the predicted behavior, *i.e.*, the positions of trough and peak are considerably shifted to about 280 and 245 nm, respectively corresponding to the positions of the absorption curve at approximately half the maximum extinction, while the midpoint of zero birefringence contribution from this band corresponds to the location of maximum absorbance.

Electric Field Dependence. The dependence of birefringence on field strengths was measured with the same DNA solution at 300 nm where the effect of dichroism is negligible. As shown in Figure 14, the optical retardation is again nearly proportional to the first order of the electric field strength (shown by filled circles) and not to the square of it (open circles). Nor is the onset of saturation of the birefringence observed at field strengths up to 7.5 kV/cm.

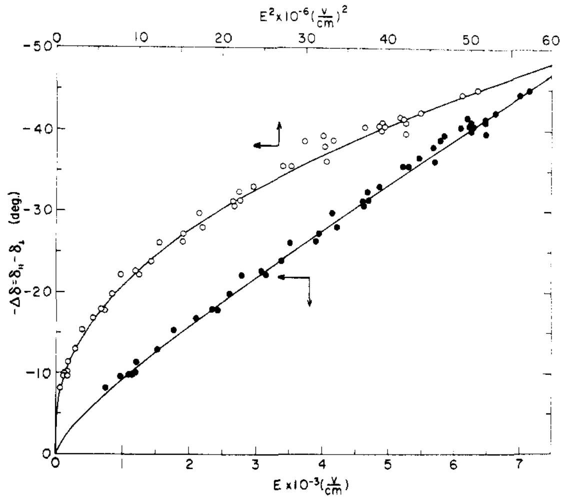

Figure 14. The electric field strength dependence of the birefringence of DNA at 300 nm. Open circles: retardation plotted against E^2; filled circles: retardation plotted against E. Solid curves are least-squares fit to the data.

Some previous workers have reported the appearance of a rather sharp and pronounced minimum in the steady-state dichroism[19] or birefringence signals[17] of DNA in Tris buffer solutions. We were unable to detect such signals at all in the 10^{-3} N NaCl solutions.

Discussion

Dichroic Spectra of DNA and DNA-Dye Complex. Neither the parallel nor perpendicular dichroism of native DNA reveal any vibrational fine structure but resemble the isotropic spectrum in 10^{-3} N NaCl (Figure 6). The specific parallel and perpendicular dichroism as well as the reduced dichroism, however, clearly show that the 260-nm absorption band is composite in nature consisting of at least three and possibly four electronic transitions between 225 and 300 nm, since the dichroism is constant only between 245 and 275 nm and decreases in the spectral regions above 290 nm, between 275 and 285 nm and between 225 and 245 nm. Between 230 and 290 nm the reduced dichroism spectrum of native calf thymus DNA as shown in Figure 8 is very close to a similar but less complete flow dichroism spectrum which contains the only comparable dichroism data available so far.[20,21a]

The fact that the specific parallel dichroism has a negative sign at all values of the field strength indicates that the angle between the apparent transition moment of the 260-nm band and the symmetry axis of the DNA molecule is larger than 54.7° (see the theoretical curves shown in Figure 15). However, the observed data (*e.g.*, $\Delta\epsilon_{\parallel}/\epsilon = -0.27$ and $\Delta\epsilon_{\perp}/\epsilon = 0.13$ at $E = 8000$ V/cm at 270 nm)

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(19) M. Shirai, *Nippon Kagaku Zasshi*, 86, 1115 (1965).

(20) A. Wada, *Biopolymers*, 2, 361 (1964).

(21) (a) A. Wada in "Quantum Aspects of Polypeptides and Polynucleotides," M. Weissbluth, Ed., Interscience, New York, N. Y., 1969, p 476. (b) The most likely candidates for this transition is that of adenosine for which an out-of-plane transition has been observed in this region in double-stranded acid poly(A) [E. Charney and J. B. Milstien, submitted for publication] and of thymine in which an out-of-plane transition has also been observed [W. A. Eaton and T. P. Lewis, *J. Chem. Phys.*, 53, 2164 (1970)]. It is possible that an in-plane transition of one or more of the bases, especially that of cytosine, which has such a transition (probably $\pi \rightarrow \pi^*$) in this region, could contribute if the bases are sufficiently tilted from the plane perpendicular to the helix axis.

(Note that within the limits of the experimental error $\Delta\epsilon_{\parallel}/\epsilon = -2\Delta\epsilon_{\perp}/\epsilon$ as required.[7]) should not necessarily be construed as indicating that the DNA base pairs are perpendicular to the double-stranded helical axis even if the transition moments are in the plane of the base pairs. The exact angle cannot be determined with native calf thymus DNA under the present experimental conditions because the saturation value $(\Delta\epsilon/\epsilon)_S$ cannot be estimated. Nor is a theoretical evaluation of $\Delta\epsilon/\epsilon$ at infinitely strong electric field possible *(vide infra)*. Whether or not the transition moments for these three or more overlapping bands all lie in the same molecular plane, which appears to make some angle less than 90° with respect to the DNA helix axis, is difficult to determine without a fairly precise knowledge of the positions, band widths and extinction coefficients for those bands.[7]

The fact that the sign of the reduced dichroism in the shorter wavelength region below 220 nm is also negative indicates that the transition(s) responsible for the absorption in this region also lies in a plane inclined at an angle more than 54.7° with respect to the DNA molecular axis or closely perpendicular to the helical axis. It is an intriguing question whether there is any optical transition in this region whose transition moment is inclined at an angle less than 54.7°. A pronounced minimum just above 220 nm is indicative of the existence of a transition whose principal component is at a considerably smaller angle with the DNA axis than that responsible for most of the intensity in the region studied. Since several of the nucleoside bases are thought to have perpendicular (to the plane of the purine or pyrimidine ring) $n \rightarrow \pi^*$ transitions in this region, it is quite reasonable to expect that this is the origin of the inflection at this wavelength.[21b] In the longer wavelength edge (>290 nm) of the 260-nm absorption band no unequivocal statement can be made to clarify the conflicting reports on the existence of an out-of-plane $n \rightarrow \pi^*$ transition in some polynucleotides.[22,23] However, in light of the steady decrease in the reduced dichroism above 290 nm, the existence of such transitions in this region cannot be ruled out.[20] Such a decrease may also result from the preferential refraction or scattering of linearly polarized light by the oriented molecules in the wings of the absorption band where the absorption is always extremely weak but variations in the refractive index are large.[24] It is interesting to note that both guanylic and cytidylic acids show absorption bands at about 280 and 228 nm at neutral pH[25] and these are the regions where the magnitude of the reduced dichroism is decreasing. The extent to which this 260-nm DNA band is a complicated average of transitions of each constituent base is still to be resolved.

The dichroism spectra of the DNA-10-Methylacridine Orange also do not show spectral features not present in the isotropic spectrum (Figures 10 and 11). Nor does the action of the electric field produce a shift of the major peak at 504 nm. However, the two shoulders at 475-480 and 440-450 nm are slightly better resolved in the dichroic spectra. These shoulders and the maximum may be assigned to a vibrational progression of an electronic transition in analogy with the isotropic spectrum of Acridine Orange[26,27] or its complex with DNA.[16] The transition moments all lie in a plane which is very likely to be the molecular plane of the bound dye; the plane makes an angle greater than 60°, at least, relative to the symmetry axis of the complex as shown in Figure 12. Whether or not the transition moment is oriented perpendicular to the axis is still unresolved due to the failure of both parallel and perpendicular dichroism to reach or approach electric field saturation ($\Delta\epsilon_{\parallel}/\epsilon = -0.26$ and $\Delta\epsilon_{\perp}/\epsilon = 0.12$ at 8000 V/cm). Nor do the present data unmask another electronic transition which may be a hidden part of the 504-nm band.[16,28] As shown in Figure 12, the reduced or specific parallel and perpendicular dichroism are essentially constant throughout the visible absorption band; recently Kurucsev and Strauss observed similar dichroic ratios for Acridine Orange in stretched poly(vinyl acrylate) films.[29] The specific parallel dichroism does show two slight humps at about 510 nm and about 470 nm. However, they are barely beyond the scattering of the experimental points and no conclusion with respect to these seems appropriate at present.

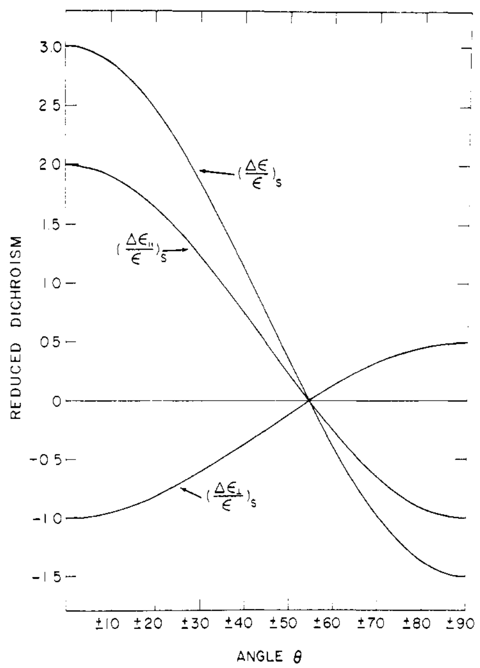

Figure 15. The theoretical curves of reduced, parallel, and perpendicular dichroism at saturating electric field strengths. Electrochroism is assumed absent. Abscissa is the angle between the transition dipole moment *m* and molecular symmetry axis. Those curves are calculated from eq 28, 29, and 30 in ref 7 for the case where $\Phi(\beta,\gamma > 0 \rightarrow 1$ as $E \rightarrow \infty$.

The molecular planes of the bound dye and the DNA base pairs appear to be parallel or nearly so, at this particular ratio of phosphate to dye. However, this result does not differentiate the subtle point which has been the subject of some controversy as to whether the dye molecules are intercalated between base pairs or are bound externally to the ionizable phosphate groups.[30-34] A more detailed investigation will be reported in due course.

In order to test if the isotropic absorption spectra of native calf thymus DNA and of the DNA-dye complex are

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(22) A. Rich and M. Kasha, *J. Amer. Chem. Soc.*, **82**, 6197 (1960).

(23) M. Gellert, *J. Amer. Chem. Soc.*, **83**, 4664 (1961).

(24) J. E. Mayfield and I. J. Bendet, *Biopolymers*, **9**, 669 (1970).

(25) P. L. Biochemicals, Inc., Circular OR-10, 1969.

(26) V. Zanker, *Z. Physik. Chem. (Leipzig)*, **199**, 225 (1952).

(27) V. Zanker, *Z. Physik. Chem. (Leipzig)*, **200**, 250 (1952).

(28) V. Zanker, M. Held and H. Rammensee, *Z. Naturforsch. B*, **11**, 789 (1959).

(29) T. Kurucsev and U. P. Strauss, *J. Phys. Chem.*, **74**, 3081 (970).

(30) D. F. Bradley and M. K. Wolfe, *Proc. Nat. Acad. Sci. U. S.*, **45**, 944 (1959).

(31) A. L. Stone and D. F. Bradley, *J. Amer. Chem. Soc.*, **83**, 3627 (1961).

(32) L. S. Lerman, *J. Cell. Comp. Physiol., Suppl. 1*, **64**, 1 (1964).

(33) D. M. Neville, Jr., and D. R. Davies, *J. Mol. Biol.*, **17**, 57 (1966).

(34) R. W. Armstrong, T. Kurucsev and U. P. Strauss, *J. Amer. Chem. Soc.*, **92**, 3174 (1970).

shifted or if their intensities are changed by the action of the external electric field, the isotropic spectra were each synthesized from the observed parallel and perpendicular dichroism data with the use of the following relationship. If no electrochromism effect results from a distortion of either the electronic or the nuclear framework, the isotropic absorbance, A_0, can be calculated in terms of the absorbances parallel and perpendicular to the external field (eq 17) and expressed as A_0^{calcd}. The observed quantities

$$A_0 = \tfrac{1}{3}(A_{\parallel} + 2A_{\perp}) \qquad (17)$$

$\Delta A_{\parallel}$ and $\Delta A_{\perp}$ are then related to A_0^{calcd} and the true isotropic absorbance A_0^{obsd} measured in the ordinary way by

$$A_0^{\text{calcd}} - A_0^{\text{obsd}} = \tfrac{1}{3}(\Delta A_{\parallel} + 2\Delta A_{\perp}) \qquad (18)$$

In the absence of electrochromism and artifacts in the measurements, this quantity must be zero, that is $A_0^{\text{calcd}} = A_0^{\text{obsd}}$. The filled triangles in Figures 8 and 12 representing these calculated absorbances (in terms of extinction coefficients) are superimposed precisely on the isotropic spectra to within the experimental error of the observations. Clearly the applied external electric field strength is too weak to induce higher order dichroic effects predicted by the theory in the preceding paper.[7] Still another conclusion can be drawn from the fact that the calculated isotropic absorption spectrum is precisely the same as the measured one in the absence of the field, namely that the secondary structure, *i.e.*, the base stacking, is not perturbed by the field because of the known dependence of the hypochromicity on this structure.

Electric Field Dependence of Dichroism of DNA and DNA-Dye Complex. The available theories of electric dichroism[7,35] and birefringence[3,4] predict that these effects should depend on the square of electric field strength at low fields for molecules with cylindrical or uniaxial symmetry in the electronic ground state, provided that the molecules have a field-independent permanent electric dipole moment along this axis only, and an electric polarizability anisotropy between this axis and either of the other two principal axes. Indeed such cases where the square law was obeyed have already been reported.[36] Interestingly the law is strictly valid only for small molecules or polymers of relatively short lengths.[37] The rigidity of the molecule is not a required condition for a quadratic dependence on field strength as long as the permanent dipole moment or the anisotropic polarizability are responsible for molecular orientation at low fields and remain independent of the external electric field.[7] However, if the molecule is flexible or the molecular conformation is affected by the field, these electric properties of the molecule may change as the strength of the field changes. This may be the case for exceedingly long macromolecules with ordered structures such as high weight helical polypeptides in nonaqueous media[3,4,8] or native DNA in aqueous solutions. Soda and Yoshioka[38] and Houssier and Fredericq[39] previously noted that the dichroism of DNA in salt-containing solutions is linearly dependent on the electric field strength at low field strengths. A further study by the latter workers[40] showed that the dependence of birefringence as well as dichroism of deoxyribonucleohistone was neither linear nor proportional to the square of the external electric field. On the other hand, Jennings and Plummer[41] recently observed a quadratic dependence of light scattering on external electric fields for native DNA in deionized aqueous solutions at extremely low field strengths.

Our results shown in Figures 8, 9, 12, and 14 also clearly demonstrate that the dichroism and birefringence of native DNA and the dichroism of the DNA-dye complex in 10^{-3} *N* NaCl deviate from an initial quadratic dependence on the electric field strengths. The plots of these electrooptical properties against a linear scale indicate that an electric field term lower than the first order is involved, if each coefficient of such terms is assumed to have the same sign (Figures 9, 12, and 14); or that the first- and higher order terms are contributory if the sign of the second-order term is just opposite to that of the linear term in *E*. Saturation values of the dichroism signals were not reached for any of the DNA solutions.[42]

If the native DNA molecule in solution were a rigid rod represented by an extended double-stranded Watson-Crick model in which the base pairs are almost perpendicular to the helical axis, the specific perpendicular and parallel dichrosim, *i.e.*, $(\Delta\epsilon_{\perp}/\epsilon)_s$ and $(\Delta\epsilon_{\parallel}/\epsilon)_s$, should be 0.5 and −1.0, respectively, at saturating field strengths as shown by the theoretical curves in Figure 15. Extrapolation from a least-squares analysis of the present data using terms in $E^{1/2}$ and E; $E^{1/2}$, E and $E^{3/2}$; and E alone predicts that the field strength at which 80% orientation would be achieved is about 28,000 V/cm. It will be shown below that these values are much larger than should be required for native calf thymus DNA with a molecular weight of about four million if it were a rigid rod oriented according to the mechanisms called for by the theoretical treatments now available.[7,8,43]

These theories predict that the saturating field or the field strength required for any value of the dichroism can be estimated, once the values of the electric dipole moment, μ, and the electric polarizability anisotropy, $(\alpha_{33} - \alpha_{11})$, of the ground state of a molecule with axial symmetry are specified. For the sake of simplicity the latter term is assumed to be zero although this is unrealistic for a long anisotropic helix-like DNA and makes the value of the required field strength abnormally *overestimated.* Values of μ for calf thymus DNA have been reported by Takashima[44] and by Jennings and Plummer[41] as listed in Table I. From these values, the values of $\beta^2 = (\mu/kT)^2E^2$ at 25° are evaluated and shown in the third column of the table. Now the field strength E_{calcd}, at which the average orientation of the molecules would be 80% or at which $\Delta\epsilon/\epsilon$ would reach 0.4, were calculated using the corresponding theoretical value of β^2 of 200. The values of E_{calcd} thus obtained are listed in Table I. We note from these values if the square law holds for DNA in 10^{-3} *N* NaCl solutions, the dichroism should approach 80% saturation in an experimentally attainable range of electric

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(35) (a) W. Kuhn, H. Duhrkop and H. Martin, *Z. Physik. Chem. (Frankfurt am Main)*, **45B**, 121 (1939); (b) W. Liptay and J. Czekalla, *Ber. Bunsenges Phys. Chem.*, **65**, 72 (1961).

(36) (a) J. B. Milstien and E. Charney, *Macromolecules*, **2**, 678 (1969); (b) T. C. Troxell and H. A. Sheraga, *ibid.*, **4**, 528 (1971).

(37) E. Charney, J. B. Milstien and K. Yamaoka, *J. Amer. Chem. Soc.*, **92**, 2657 (1970).

(38) T. Soda and K. Yoshioka, *Nippon Kagaku Zasshi*, **87**, 1326 (1966).

(39) C. Houssier and E. Fredericq, *Biochim. Biophys. Acta*, **88**, 450 (1964).

(40) C. Houssier and E. Fredericq, *Biochim. Biophys. Acta*, **120**, 113 (1966).

(41) B. R. Jennings and H. Plummer, *Biopolymers*, **9**, 1361 (1970).

(42) Some preliminary data on sonicated calf thymus DNA, in which the quadratic field dependence is observed has been reported: E. Charney and K. Yamaoka, *XXIII Int. Congr. Pure Appl. Chem., Macromol. Prepr.*, **1**, 252.

(43) M. Matsumoto, H. Watanabe and K. Yoshioka, *Sci. Papers Collect. Gen. Educ., Univ. Tokyo*, **17**, 173 (1967).

(44) S. Takashima, *J. Mol. Biol.*, **7**, 455 (1963).

Table I

	$\mu \times 10^{14}$, esu	$\beta^2 = (\mu/kT)^2E^2$	E_{calcd} (80% Orientation), V/cm
Takashima[44]	3.2	0.605	5,500
	1.5	0.133	11,600
Jennings and Plummer[41]	7.5	3.33	2,300

field of 2-12 kV/cm, *notwithstanding the total neglect of the contribution of the polarizability anisotropy* to the orienting torque. The discrepancy between the values of approximately 28,000 V/cm obtained from extrapolation of the measured dichroism and the values in Table I is new evidence for the inherent flexibility of the native calf thymus DNA helix.[45] It should be noted by way of caution that in the strict sense the electric field strength considered here should not be the applied field but the effective one in the vicinity of the molecule concerned.[46]

Electric Dichroism *vs.* Birefringence inside the 260-nm Band of DNA. A method for determining the phase difference or optical retardation by measuring the intensity of light transmitted through crossed polarizers introduces a serious error when the measurement is carried out inside a dichroic absorption band as clearly demonstrated in this work for the first time (Figure 13). To make the necessary corrections, the effect of linear dichroism must be taken into account. As can be seen from the figure, the anomalous dispersion of the linear birefringence calculated from the observed data with the aid of eq 16, which ignores the effect of the dichroism, is grossly altered in shape and in the positions of trough and peak (the magnitude remains unchanged). Thus, the electric birefringence dispersion data inside an absorption band determined with any apparatus similar to the present one must be used with caution for quantitative considerations, unless a complete set of the corresponding parallel and perpendicular dichroism data is obtained under exactly the same conditions for necessary corrections. From these results, previous work on the anomalous birefringence dispersion of the dye-polymer complexes should be considered only from a qualitative point of view.[47-49] An infrared Kerr effect study[10] also ignored the contribution from dichroism, but the degree of orientation achieved with the small molecule in this study probably was insufficient to have a pronounced effect.

Although dispersion of the electric birefringence, as such, is a sensitive method of determining the relative orientation of a chromophore, the data corrected for dichroism is inevitably less accurate. This reduces the potential usefulness of the birefringence technique unless some methods, which do not require such corrections, are developed. However, a very weak birefringence can still be observed with the use of a phase-retardation plate under conditions where the dichroism signal is too weak to be detected. Therefore, the birefringence method may be the method of choice for molecules with small dipole moments and polarizability anisotropy such as globular proteins or oligomeric polymers. Some efforts are now being made to measure the true anomalous birefringence dispersion inside an absorption band without having recourse to electric dichroism measurements.

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(45) This has long-been indicated by hydrodynamic and light-scattering measurements. See, *e.g.*, the review by V. N. Tsvetkov in "Newer Methods of Polymer Characterization," B. Ké, Ed., Interscience, New York, N. Y., 1964.

(46) C. J. F. Böttcher, "Theory of Electric Polarization," Elsevier Publishing Co., Amsterdam, 1952, p 174.

(47) J. C. Powers, Jr., *J. Amer. Chem. Soc.*, 88, 3679 (1966); 89, 1780 (1967).

(48) J. C. Powers, Jr., and W. L. Peticolas, *J. Phys. Chem.*, 71, 3191 (1967).

(49) D. F. Bradley, N. C. Stellwagon, C. T. O'Konski, and C. M. Paulson, *Biopolymers*, 11, 645 (1972).

Studies of Polymers by Nuclear Magnetic Resonance at Magic Angle Rotation. III. Poly(γ-benzyl L-glutamate) in Solution†

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ABSTRACT: Nmr spectra of solutions of poly(γ-benzyl L-glutamate) in $CDCl_3$ have been measured with magic angle rotation (MAR) at frequencies of 300-5000 Hz. In a solution of liquid crystal type, high-resolution spectra of the polymer in the α-helical form have been obtained. Bands of various proton groups in the side chain appear in the spectrum gradually with increasing spinning speed, but narrowing of the bands of protons on the helix backbone could not be achieved. In the narrowed lines, the rotationally invariant residual line widths are a function of frequency and form of rapid anisotropic motions of the respective groups.

In our preceding papers[1-3] it has been shown that, by rotation of the sample about an axis inclined at the so-called magic angle $\theta = 54.7°$ with respect to the direction of the static magnetic field H_0, and at a frequency comparable to the line width of the static sample ($\omega_r \gtrsim \delta\omega_{st}$), a significant line narrowing can be obtained in proton nmr spectra of some solids and heterogeneous systems normally broadened by static dipolar interactions. In heterogeneous solid-liquid systems, regular high-resolution spectra of the liquid phase can be obtained.[1,3] In the spectra of solid polymers magic angle rotation (MAR) usually leads to considerable line narrowing,[1] but the "high-resolution limit" has not been reached, because the residual line width is limited by the frequency of internal motion.[2]

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† Part II: D. Doskočilová and B. Schneider, *Macromolecules*, 5, 125 (1972).

(1) D. Doskočilová and B. Schneider, *Chem. Phys. Lett.*, 6, 381 (1970).

(2) B. Schneider, H. Pivcová, and D. Doskočilová, *Macromolecules*, 5, 120 (1972).

(3) D. Doskočilová and B. Schneider, *Macromolecules*, 5, 125 (1972).