Contribution of Light Scattering to the Circular Dichroism of Deoxyribonucleic Acid Films, Deoxyribonucleic Acid–Polylysine Complexes, and Deoxyribonucleic Acid Particles in Ethanolic Buffers†

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ABSTRACT: The contribution of scattering to the circular dichroism (CD) of DNA films with twisted structures, DNA–polylysine complexes, and condensed DNA aggregates in ethanolic buffers of defined salt concentrations has been studied by the use of novel measuring techniques. These techniques include fluorscat cuvettes, fluorescence-detected circular dichroism (FDCD) methods, backscattering capturing devices, and beam-mounted goniometer detectors. The result of the experimental measurement is that DNA films can be made which have very large ellipticities or CD at sharp specific wavelengths. The sign of these ellipticities is related to the handedness of the twists, with a right-handed twist producing large positive rotations and a left-handed one producing negative rotations. The film shows nodal angles at which the interaction with light is minimal. The scattering patterns of both films, DNA–polylysine particles and DNA–EtOH condensates, show that the main interaction is light scattering produced by a resonance phenomenon similar to that produced in cholesteric liquid crystals and twisted-nematic liquid crystals. From this result we propose that the so-called ψ-type CD spectrum is a manifestation of a side-by-side packing of DNA molecules with a long-range twisting order whose helical parameters match the helical parameter of circularly polarized light at specific resonance or critical wavelengths. Application of the Bragg law for cholesteric liquid crystals gives the periodicity of the long-range ordered structures.

As can be seen in the first paper in this series (Reich et al., 1980), the perturbations due to scattering of the CD signals on a variety of particles can be divided into two general types: (a) that signal which can be corrected by recapturing the light deviated away from the detection system and (b) that CD signal which is not corrected by increasing the efficiency of light collection and which may even be *enhanced* by increasing the envelope of light capture and/or the efficiency of measurement of the CD signal about the scattering particle.

The first type of perturbation is the best understood (Holzwarth et al., 1974; Gordon, 1972; Gordon & Holzwarth, 1971). Reasonable approximations to certain types of optically active particles by the application of Mie scattering theory of a sphere have had a measure of success in explaining the absorbance spectrum and some of the so-called Duysens (1956) flattening manifested by large membranes (Schneider & Harmatz, 1976). However, it was found in the case of bacteriophage T2 that the above theory was not successful in predicting the CD of the condensed nucleic acids inside the head of the virus (Holzwarth et al., 1974). In fact, the CD scattering components of the intact virus as measured by Dorman & Maestre (1973) show properties very similar to those measured in the optical activity dispersion curve of cholesteric liquid crystals (Holzwarth et al., 1974).

The optical properties of liquid crystals with a well-defined chiral structure (i.e., cholesteric liquid crystals and twisted-nematic liquid crystals) are indeed remarkable in their nature. They have been extensively studied [see de Gennes (1974) and Chandrasekhar (1977) for excellent treatises on the analysis of the optical properties of liquid crystals], and since these optical properties are produced by a phenomenon of macroscopic dimensions, they can be easily analyzed by the use of macroscopic dielectric tensors in combination with Jones matrix calculus and Stokes vectors [see Shurcliff (1962) and Chandrasekhar (1977)]. We shall mention here some of the most pertinent properties.

(1) Cholesteric liquid crystals have bands at critical wavelengths at which strong interactions occur with impinging light of the correct polarization. That is, a cholesteric liquid crystal of right-handed helical structure will interact strongly with a right circularly polarized wave impinging upon it if the periodicity of the wave matches that given by a Bragg reflection law, to wit

$$n\lambda = 2L \cos r' \qquad (1)$$

(de Gennes, Chapter 6, 1974) where n = order, λ = wavelength, L = reagent period, and r' = internal angle of refraction.

(2) If at that wavelength there exist chromophores absorbing light, the phenomenon of anomalous transmission occurs (Nitayananda et al., 1973; Suresh, 1976) in which an apparent excess transmission or loss of absorbers is measured. This is analogous to the Borrmann effect (1941) for X-ray diffraction when a crystal is set for Bragg reflection.

(3) The polarization of the reflected waves is the same as that of the impinging wave, which is the opposite case to that of ordinary reflection in which there is a phase shift of 180°. Thus, for a cholesteric array at the critical resonance wavelength, the scattered light has the same sense of polarization as the incoming wave. Moreover, it reflects the helical sense of the scattering structure, and it is related to the pitch by eq 1. In this property it also mimics the scattering behavior of an array of helical wire antennas for circularly polarized radio waves (Krauss, 1950). These properties were used by Tinoco & Freeman (1957) to model the interaction of light with optically active chromophores by the use of arrays of wire coils and a radar transmitter with detector in the 1.4-cm wavelength range.

(4) The most remarkable property of all is the strength of the interaction. The rotational strengths measured for cholesteric liquid crystals or twisted nematics are 10^4 times larger than that of the optically active components. This is because of the very strong resonance coupling with the light beam at

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the critical wavelength. Thus, the large magnitude of the CD signals provides a strong clue to the explanation for the so-called ψ-type spectra measured in DNA aggregates or particles (Jordan et al., 1972).

$\psi(\pm)$-Type CD spectra have been reported with very large specific circular dichroism in films of DNA by Tunis-Schneider & Maestre (1970) and by Brunner & Maestre (1974). Magnitudes for $\epsilon_l - \epsilon_r = -300$ for films of poly[d(A-T)·d(A-T)] and values as large as −70 for films of calf thymus DNA have been measured by these workers. Large values have also been reported for polylysine–DNA aggregates ($\Delta\epsilon = -28$) and poly(uridylic acid) and polylysine complexes or aggregates ($\Delta\epsilon = +120$) by Carroll (1972). Reich et al. (1980) showed very large values for DNA particles produced by specific concentrations of salt–EtOH buffers.

What is most remarkable about these nucleic acid complexes, films, and aggregates is that the large values are *not* corrected by large-angle integrating sphere methods, and in the case of the DNA–EtOH particles, the magnitudes are substantially increased. The scattering components of the CD of the particles that are correctable by FDCD or fluorescent methods also show remarkable directional properties which are not typical of the ordinary scattering behavior of nonoptically active particles and of some optically active particles. For DNA–EtOH–salt condensates or particles, the main CD (correctable) scattering components are at right angles to the incoming light beam and also in the *back* direction with very little measured in the forward scattering cone.

All the above-mentioned experimental characteristics lead us to believe that what we are measuring are manifestations of optically active behavior of long-range order of the type seen in cholesteric or twisted-nematic liquid crystals or, alternatively, that of helical antennae arrays.

Thus, we first attempted to develop cholesteric or twisted-nematic films of DNA and then proceeded to measure the reflection or resonance phenomena or the scattering patterns by novel experimental techniques. In the next section we will describe in detail how we measured every directional component of the differential light scatter of DNA films of defined helical superstructure, of DNA–polylysine complexes of varying (DNA/polylysine) ratios, and of ethanol-condensed DNA, or particles, of extremely large optical rotations or dichroism.

We shall show by the use of these techniques and materials that ψ-type spectra, both positive and negative, are a manifestation of superorganization of the DNA in these particles, films, and aggregates. Moreover, these CD signals occur at specific wavelengths reflecting the repeat periodicity of the helical superstructures, and such periodicities are related to the maximum and minimum CD signals by some type of reflection Bragg scattering law.

Techniques and Methods

We shall describe in some detail the variety of techniques used in measuring the optical interaction of films, DNA–polylysine particles, and ethanolic particles. Some of these techniques have been reported previously, having been used in the measurement of scattering optically active particles (Dorman & Maestre, 1973; Dorman et al., 1973). It is important to understand *how* and precisely *what* each of these techniques is measuring and how the signal from the CD machine must be further processed to obtain the true intensity and polarization values for the light after interaction with the scattering object.

Circular dichroism was measured by the Grosjean & Legrand (1960) technique. This technique uses the modulation of linear polarized light by a quarter-wave plate to which is applied an oscillating variable voltage (Pockels' cell or electrooptic modulator), thereby rotating its optical axes by 90°, back and forth, producing a positive or negative 90° phase shift on the x,y components of the incoming light beam. The result is that circularly polarized light is produced, first with one sense of polarization, followed by the opposite sense (i.e., left circularly polarized light followed by right circularly polarized). There are several ways of producing this effect including mechanical rocking of the $\lambda/4$ plate or the use of piezoelectric modulators (Morvue modulator). The circularly polarized light of intensity I_0 interacts with the material under investigation, and after the interaction, a quantity I is measured. The usual circular dichrograph, following the design of Grosjean & Legrand (1960), obtains by electronic means a ratio of intensities

$$\frac{I_l - I_r}{I_l + I_r} = \frac{\text{(ac component)}}{\text{(dc component)}} = \text{signal} \tag{2}$$

If the material in question obeys a Beer–Lambert absorption law for each polarization, we have for

$$I_l = I_{0l}e^{-c\epsilon_l d} \qquad I_r = I_{0r}e^{-c\epsilon_r d} \tag{3}$$

and signal = $k \tanh (\epsilon_l - \epsilon_r)$

$$\text{signal} = k(\epsilon_l - \epsilon_r) \text{ for ellipticities of the order of 5° or less} = k\Delta\epsilon \tag{4}$$

where ϵ_r = extinction coefficient for right circularly polarized light and ϵ_l = extinction coefficient for left circularly polarized light. The above equation implies pure absorption phenomena. No scattering (anisotropic or otherwise), fluorescence, or phosphorescence is assumed, and the measuring instrument is perfect (i.e., no stray light, linear birefringence in optics or detectors, or linear dichroism or circular dichroism in the optics or detectors is assumed).

For the case in which a particle large enough to produce long-range effects among the interacting chromophores, for a given range of wavelength, the above equations must be modified to include these perturbations:

$$\text{signal} = k\frac{(I_l - S_l) - (I_r - S_r)}{(I_l - S_l) + (I_r - S_r)} \tag{5}$$

where k = instrument constant, S_l = scattered left circularly polarized light, and S_r = scattered right circularly polarized light.

The terms S_l, S_r can be defined formally to include the following: (a) Scattered light deviated isotropically from the main direction of the beam (ordinary scattering, $S_l = S_r, S_l > 0$). (b) Scattered light deviated differentially (i.e., differential circular dichroism scattering). Linear dichroic scattering can also occur and can be of importance in film work later. $S_l \neq S_r$, S = function of orientation angles with respect to light beam. (c) Stray light from cuvette window reflections, wall reflections, dust in solutions and in cuvette windows, and reflections from the optics contributed. (d) The signal can also include Beer–Lambert contributions from such particles whose effective extinction is almost total, in which case the effect is manifested as a Duysens flattening distortion for the circular dichroism signal. This has been seen in membranes (Schneider, 1973) whose red blood cells were hemolyzed (Gitter-Amir et al., 1976) and in crystals of protein (in this last case reversibly) (W. Chiu and M. F. Maestre, unpublished results). (e) Most importantly, the signal can include enhancement scattering due to a manifestation of anomalous transmission: $S_l \neq S_r$ and S_l or S_r having a negative value so as to make it appear that less absorption occurs for a given

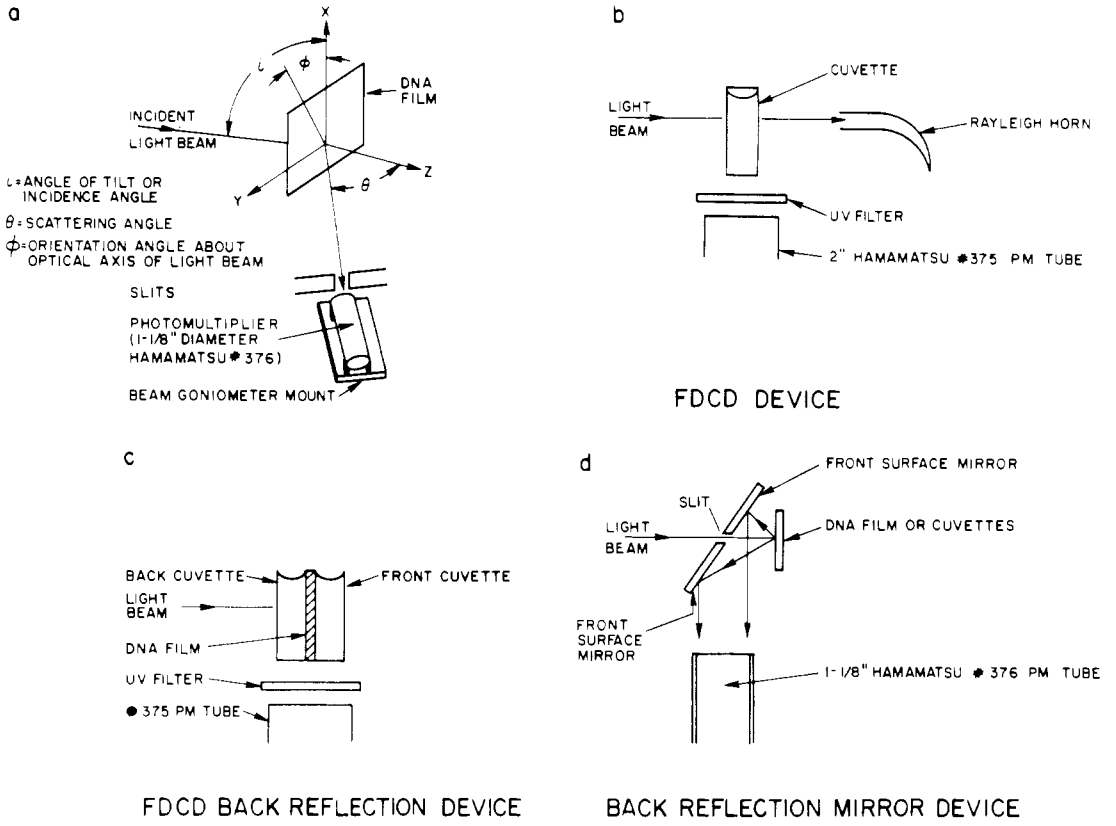

FIGURE 1: Schematics of the differing techniques and devices used in the measurement of the CD scattering components of films, DNA–polylysine particles, and DNA–EtOH condensates. All these devices were used in a modified Cary 60 dichrograph.

set of chromophores (i.e., anisotropic hypochromicity) for a particular polarization and region of the spectrum [see Chandrasekhar (1977)].

The above equations[5] describe the case in which the detection system is centered about the incoming light beam and also measures the incoming light plus all scattering components that lie within its acceptance measuring cone.

If the technique allows measurements outside the main light beam (i.e., CD as a function of scattering angle by a goniometer detector system), the terms I disappear and we have now

$$\text{signal} = k\Delta\theta \frac{S_l(\theta) - S_r(\theta)}{S_l(\theta) + S_r(\theta) + \text{stray light components}} \quad (6)$$

where $\Delta\theta$ = angle of acceptance of the detection system. (Note: At a given angle θ, the total scattering $S_l(\theta) + S_r(\theta)$ can be very small, so that the stray light can make quite a large contribution. Conversely, if both stray light and total scattering are small at a given angle θ, we may have a very large apparent CD at that angle. Thus, the interpretation of these types of measurements should be done with caution and should be checked by differing techniques as we did in our measurements.)

In addition to (a–e), for the measurement of the CD of films and the scattering CD components, the equations now become functions of the angles of incidence i (tilt angle), the angle of internal refraction r, and the orientation angle *about* the axis of the incoming light beam as shown in Figure 1a. It has been shown by Tunis-Schneider & Maestre (1970) that a film with linear orientation will introduce an apparent circular dichroism component to the intrinsic CD as given by

$$CD_{apparent} = CD_{intrinsic} - 0.298p \cos 2\phi \quad (7)$$

where p = linear dichroism of DNA and ϕ = angle of rotation about the optical axis.

Therefore, it is important when measuring the CD of films that have been manipulated in their manufacture to check for possible linear dichroism components by rotating the film assembly about the optical axis.

Nomenclature and Description of Techniques

Small-Angle Acceptance Circular Dichroism ("*Normal CD*"). Acceptance angles range between 3° and 5°. This is the standard CD machine configuration.

Large-Angle Acceptance CD. This is usually measured with a large 2-in. diameter end window PM tube (Dumont No. 2706 or Hamamatsu R375) especially selected for low birefringence artifacts. The angle of acceptance is increased by bringing the tube close to the cuvette [see Dorman et al. (1973) for a complete discussion of this technique].

Fluorescence Cuvette Integrating Methods. As described by Dorman et al. (1973), these are two cuvettes, one inside the other. The outer one is filled with a fluorescent solution whose main absorbance band covers the region of the spectrum of interest, in this case sodium salicylate in absolute ethanol. The mechanism of correction utilizes the fact that all light that is not absorbed in the inner chamber eventually hits a fluorescent molecule in the other chamber. What the photomultiplier sees is the fluorescence emission due to excitation by this light. The fluorescence cuvette covers most of the scattering envelope except for a region toward the back where the light beam impinges upon the first window of the inner cuvette containing the material of interest.

Fluorescence-Detected Circular Dichroism (FDCD) (See Figure 1b). As shown in the preceding article, this technique, properly used, corrects for all the differential scattering including the backscattering cone. It is probably the most efficient means of recovering light scattered in all directions. An interesting modification of this method is the one described in Figure 1c where a DNA film is sandwiched between two

fluorescence cuvettes, a back cuvette covering the backscattering cone and a front cuvette measuring the front scattering cone. This technique allows the *individual* and *separate* measurement of these two regions of the scattering envelope. This technique was used to measure the backscattering lobes of the DNA films, the DNA–EtOH condensates, and the DNA–polylysine aggregates.

A similar technique is shown in Figure 1d where a front-surface mirror recovers a large part of the backscattered light and reflects it into the detector.

The beam goniometer mounted detector (Figure 1a) provides information on the CD scattering as a function of scattering angle. The scattering acceptance angle is defined by the width of the slits in front of the photomultiplier tube.

Vertical Assembly and Tilt Angle Device. The manufacture of films that are extremely wet implies that the film has to lie parallel to the horizontal until it is reasonably rigid. To measure the CD of these wet films and the tilting angle studies, we used the instrument developed for CD film studies by Maestre (1970). This device allows the rotation of the film assembly about the light beam (rotation of ϕ, Figure 1a) in order to study linear dichroism artifacts and the detection of "null" scattering angles i by tilting.

Preparation of the Films

This is an art form, and practice makes perfect. For example, calf thymus DNA is placed in a totally dehydrated lyophilized form on top of the bottom fused silica window. Distilled water or buffer is added drop by drop until all the DNA is submerged under a solution droplet. After the DNA fibers have swollen and gone into solution to form a very viscous liquid, they are covered very gently by the top quartz plate and then pressed down with the proper twist (counterclockwise or clockwise). This twist is small—only about 45° to 90° rotation initially. Then the film is placed in the vertical assembly, and its CD is measured. Usually the rotations are so large that the maxima of the CD peaks cannot be in the Cary 60 CD instrument (2° maximum scale). The film is then squeezed some more to reduce the thickness enough to bring the measurement on scale. After the CD is measured, the twist is then reversed until an opposite CD is obtained (usually about 90°–180° twist) and so forth. The films usually have an optical density at 260 nm between 0.3 to 0.7. Usually about four to five reversals can be done before the films become so disoriented that one observes the ordinary CD of DNA (conservative CD spectra). One must guard against introducing linear orientation components, and this is checked by rotating the whole film assembly by 90°.

Preparation of the T7 Phage DNA–Polylysine Scattering Complexes: $\psi(-)$-Type Particles

0.85 M and 1 M Salt. A solution of T7 phage DNA was obtained by phenol extraction from intact T7 bacteriophage and then dialyzed into 1.5 M NaCl and 0.01 sodium cacodylate, pH 7.0. Poly(L-lysine hydrobromide) (VIIB) was purchased from Sigma Chemical Co. This was reported to have a molecular weight of 32 000 and a dp of 150 by the supplier. A sample of the polylysine was submitted to the microanalytical laboratory of the Chemistry Department, University of California, Berkeley, for an elemental analysis in order that accurate solution concentrations of the polynucleotide could be obtained. Solutions were made up in 1.5 M NaCl and 0.01 M sodium cacodylate, pH 7.0, and added to the above DNA solution so that a final 1:1 ratio of nucleotide to lysine residue was obtained. In 1.5 M salt, polylysine and DNA do not form particles, while at lower salt concentrations in which the two species do condense, a 1:1 ratio of lysine to DNA base is observed (Shapiro et al., 1969). The DNA–polylysine mixture was then dialyzed into 1 M NaCl and 0.01 sodium cacodylate, pH 7.0. Shapiro et al. (1969) reported that from 0.85 M to 1.0 M NaCl, the salt concentration was at an optimum for the formation of reversible particles having $\psi(-)$ DNA character.

0.15 M Salt Solutions. Two solutions of T7 DNA–polylysine in 0.15 M NaCl and 0.01 M sodium cacodylate, pH 7.0, were prepared by using differing methods. Solution 1 was prepared by dialyzing the DNA–polylysine mixture into 0.85 M NaCl and then into 0.15 M NaCl and 0.01 M sodium cacodylate, pH 7.0. Solution 2 was prepared by dialyzing the DNA–polylysine mixture into 1.0, 0.7, 0.4, and finally 0.15 M NaCl and 0.01 M sodium cacodylate, pH 7.0. Both types of particles showed the CD characteristics of $\psi(-)$-type particles.

Escherichia coli DNA–Polylysine Complexes. E. coli DNA was obtained from Worthington Biochemicals. A solution was made up in 1.5 M NaCl and 0.01 M sodium cacodylate, pH 7.0, and run through a 23-gauge needle 11 times in order to reduce the size of the DNA molecules. The polylysine was then added so that a final 1:1 ratio of lysine to nucleotide was obtained. $\psi(-)$ DNA particles were produced by dialyzing the polylysine–DNA mixture vs. 1.0 M NaCl and 0.80 M NaCl/0.01 M sodium cacodylate, pH 7.0. In this case, the salt concentrations were the same as those of Shapiro et al. (1963).

E. coli DNA Ethanolic Condensates. E. coli DNA solution was placed in 80% ethanol-buffered solutions as reported previously (Reich et al., 1979). This particular preparation was highly concentrated, however, with an optical density value of 10 at 260 nm, prior to the addition of the ethanol. With this method most of the DNA is precipitated. The precipitated material is concentrated in a high speed centrifuge after the ethanol is evaporated off under vacuum. The DNA is then redissolved in buffer, and the addition of ethanol to 80% w/w is repeated. In this manner, DNA ethanolic $\psi(+)$ particles with very large positive ellipticities are obtained.

Computations of Scattering Components. Several modifications have to be introduced in the FDCD equations to allow computation of the backscattering or resonance reflection intensities for each polarization. An appendix (see paragraph at end of paper regarding supplementary material) shows the equations for the following cases: (i) film with no scattering components gives with both cuvettes filled in (Figure 1c)

$$\theta_F = (14.32)(2.303)\Delta A/(10^A + 1)$$

$$\Delta A = \frac{\theta_F(10^A + 1)}{32.98}$$

(ii) film scattering all in the back direction, both cuvettes filled in (Figure 1c)

$$\Delta A = \frac{\theta_F(10^A + 1)}{32.98}$$

(iii) film with scattering all in the back direction with the back cuvette full of fluorescer (Figure 1c)

$$\theta_F = -14.32\frac{\Delta A_s}{A_D}(10^{-A_F} - 10^{-A})$$

$$\Delta A_s = \frac{-\theta_F A_D}{14.32(10^{-A_F} - 10^{A})}$$

where ΔA_s = differential backscattering component or resonance reflection (see supplementary material), A_D = absorption of film, A_F = absorption of fluorescer in back cuvette, and A

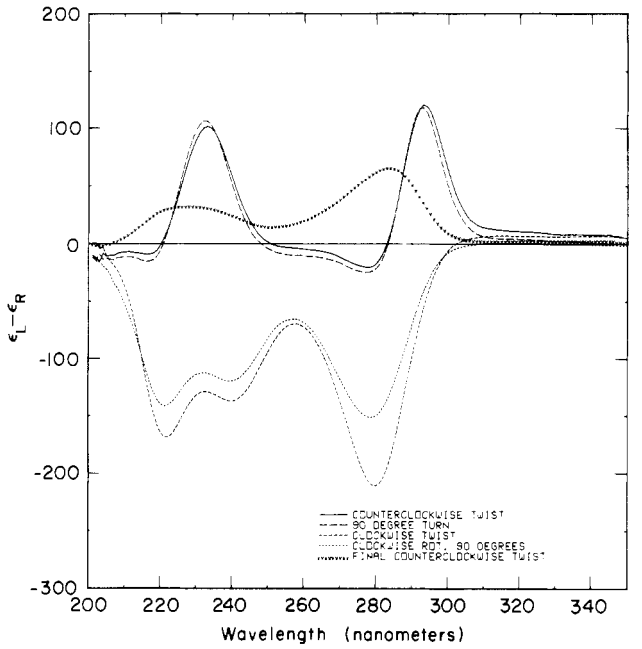

FIGURE 2: CD of DNA films measured in a vertical CD assembly as a function of twist of film and orientation of film about the optical axis of the light beam. Twisting occurred in the sequential order described in the curve labels.

= total absorption and scattering extinction of complete system. These are the equations used in computing the components obtained by the method described in Figure 1c.

Results

Film Experiments. Figures 2–6 show the results of a variety of techniques employed in measuring the interaction with circularly polarized light. Figure 2 displays the circular dichroism as a function of twist. The magnitudes at the maximum are $\Delta\epsilon$(right-handed twist) = +120 at 293 nm and +100 at 233 nm. When the film is twisted clockwise (i.e., a left-handed twist), we observe $\Delta\epsilon$(left-handed twist) = −210 at 280 nm and $\Delta\epsilon$ = −169 at 222 nm. These are enormous values for the CD of DNA. DNA in solution has values on the order of $\Delta\epsilon$ = +2.2 at 275 nm and $\Delta\epsilon$ = −3.3 at 245 nm. The CD is shown to be reversed once more by again twisting counterclockwise. It should be noticed that the positions of the maxima and minima shift toward lower wavelengths as the films dehydrate with time or are squeezed tighter. This could be interpreted as a change in some periodic parameter in the superorganization introduced by the twisting. It should be also noted in Figure 2 that rotating the whole assembly by 90°, a test for linear dichroism artifacts (eq 7) that changes the signal shape little and only affects the values by lower magnitude effects, is probably caused by nonuniform thickness of the film.

Figure 3 shows the CD of a film sandwiched between the two cuvettes as shown in Figure 1c. The direct CD measured showed values of $\Delta\epsilon$ = −260 at 287 nm and −280 at 232 nm. When the backscattering cone was measured by the use of the FDCD technique by placing the α-naphthylamine fluorescer in the back cuvette, we obtained a remarkable backscattering CD signal with $\Delta\epsilon$ = −120. The front scattering measured by FDCD still gave a large value (−160). For the peak at around 230 nm, the value either changed sign or was considerably reduced in magnitude. Unfortunately, in this region the α-naphthylamine used in the FDCD measurement absorbs strongly so the signal to noise ratio is poor and consequently the measurement is not trustworthy. When a similar measurement of the backscattering cone is made for a different DNA film with a counterclockwise twist ("positive CD") by the mirror technique, we again obtained similar behavior. This film had values of $\Delta\epsilon$ = +540 at 284 nm and +321 at 227 nm measured with regular CD (Figure 4).

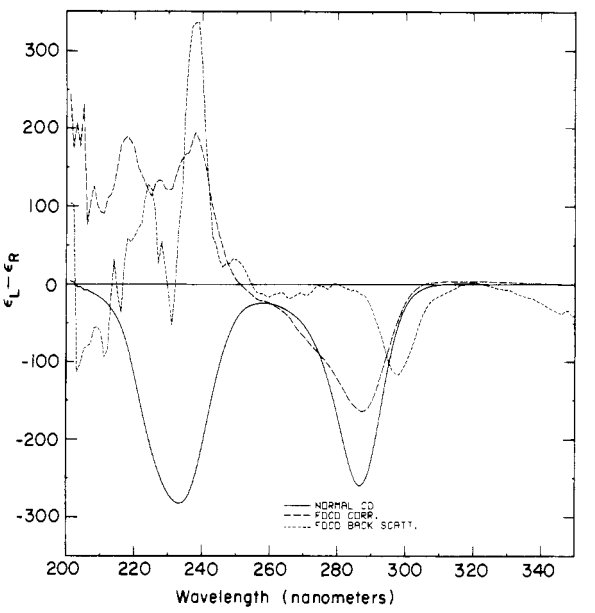

FIGURE 3: DNA film with a clockwise twist producing a negative ψ(−) CD spectrum. This film was produced between two cuvettes as described in Figure 1c to enable measurement of "normal" CD. A forward cone is a forward cell full of fluorescer in the FDCD mode, and a back cone is a back cell full of fluorescer in the FDCD mode. Note that scattered light is equivalent to transmitted light, and since the CD instrument measures $(T_r - T_l)$, the curve labeled FDCD backscatter is $(A_{sr} - A_{sl}) = -A_s$ as determined by the equations in the supplementary material.

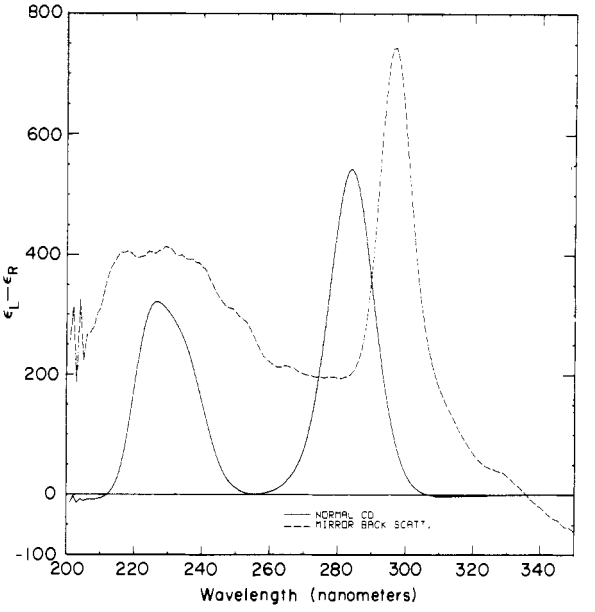

FIGURE 4: DNA film with counterclockwise twist measured by normal CD and the back mirror device as in Figure 1d. Notice the polarization remains essentially the same throughout the spectrum.

The back-reflection-mirror measurement shows a value of $\Delta\epsilon$ = +742 at 297 nm and +414 in the 220–230-nm region. One must remember that these magnitudes are only for true scattering components and can be exaggerated by the denominator in eq 6. It is obvious, however, that in these cases, at least for the CD signals in the 270–290-nm region, the backscattering components have the same polarization and

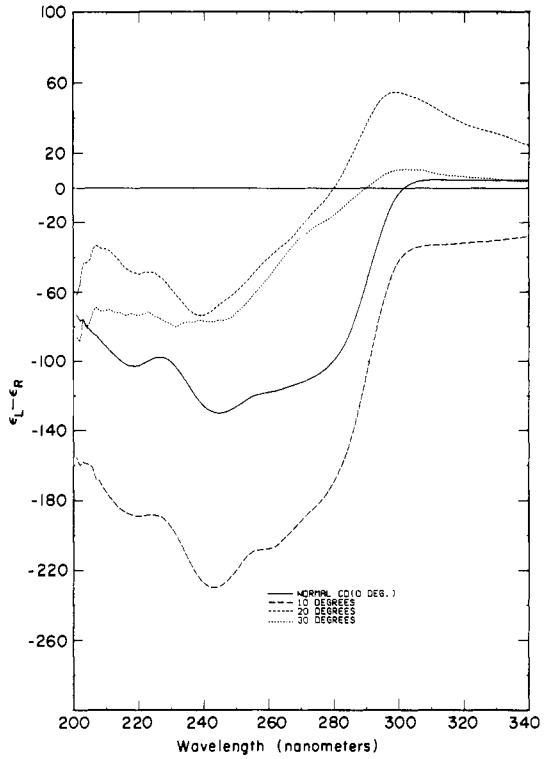

FIGURE 5: DNA film with clockwise twist as a function of scattering angle. The goniometer device is sketched in Figure 1a.

approximately the same order of magnitude as the signals measured in the forward direction.

The behavior of the scattering components for the DNA film as a function of the scattering angle is shown in Figure 5. The CD shows a complicated behavior as the angle increases, but in the region of the large minimum the sign remains negative. Thus in that region of the spectrum, the scattered light shows essentially the same polarization as is measured in the "ordinary" CD measurement. Of interest is the CD scattering in the nonabsorbing regions, $\lambda > 300$ nm. These CD "tails" into the visible have been interpreted as loss of light of a particular polarization by deviation away from the detector (Dorman & Maestre, 1973). For scattering angles $\theta = 0°$ to $\theta = 10°$, we see that there is a change in sign, and this indicates that scattered light of the opposite polarization is being measured. Thus in regions of the spectrum away from the absorbance bands, the CD scattering can be a complicated function of the scattering angle. Similar behavior will be seen later in the CD scattering of DNA–polylysine aggregates.

Figure 6 is a study of the variation of "normal" CD measurement as the angle of incidence of the light beam impinging upon the films is altered. These measurements were done to see if the behavior of the twisted DNA films in any way mimicked that of twisted-nematic or cholesteric liquid-crystal structure. For this particular film we found that a minimum in the magnitudes of the CD peaks occurred at about a ±20° tilting angle. This implies that there are certain directions in which "nulls" occur (i.e., the interaction with circularly polarized light is a minimum or is *equal* for both polarizations).

The above results have shown that the CD of twisted DNA films has very large magnitudes (i.e., large interactions), is dependent on the sense of the twist of the film, and can be reversed by reversing the sense of the twist and that the CD signal and CD scattering signal are very sensitive to the orientation of the film vs. the light beam and/or scattering angle of the light after interaction. In analogy to the optical properties of liquid crystals (cholesteric or twisted nematics),

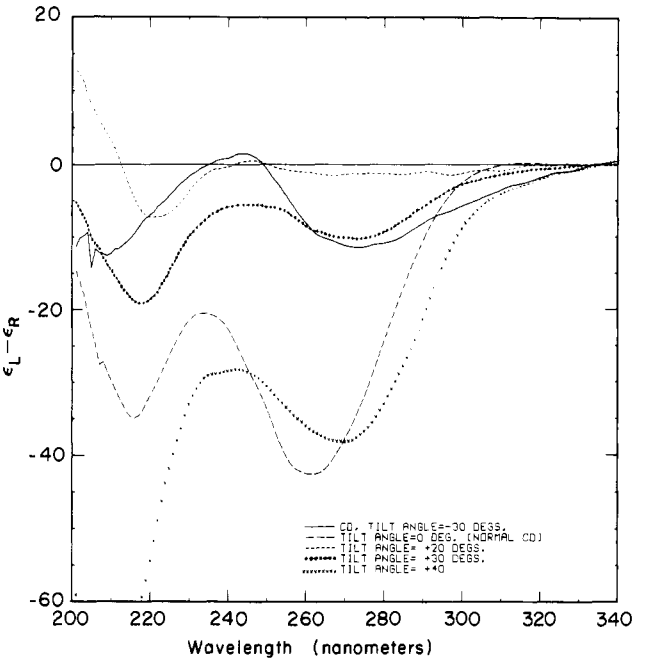

FIGURE 6: DNA film as a function of changing incidence angle of light beam. This is measured in the vertical mode device.

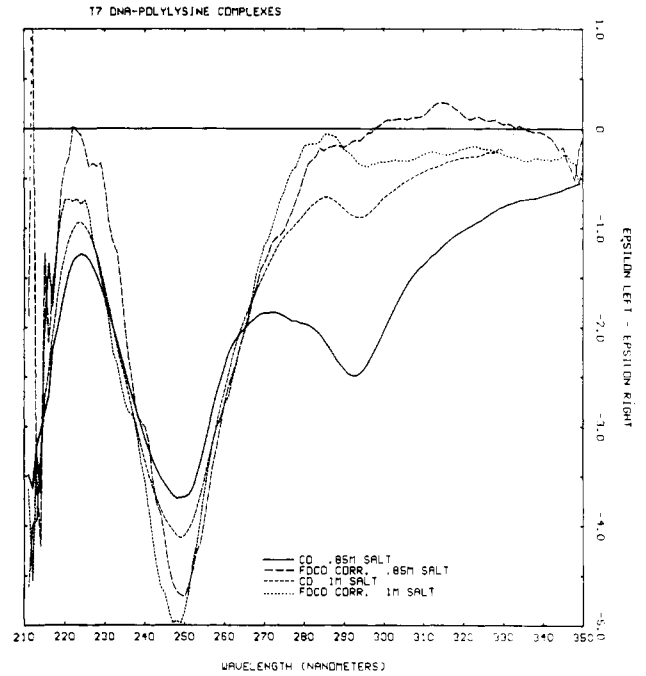

FIGURE 7: T7 phage DNA–polylysine complexes produced in different salt concentrations. The FDCD correction is achieved by the device shown in Figure 1b.

we propose the interpretation that the CD signals are a result of the long-range order and organization of DNA closely packed in films or aggregates.

DNA–Polylysine Aggregate Experiments

Figure 7 shows the CD scattering behavior of T7 phage DNA–polylysine and *E. coli* DNA–polylysine complexes at different stages of aggregation. Figure 7 shows attempts at correction of the CD spectrum by FDCD methods. Presumably FCDC techniques as shown by Reich et al. (1980) will correct for all the scattering contribution that involves only deviation of the light away from the detector acceptance cone. It is shown that away from the negative CD band at 245–250 nm the FDCD correction does a fair job of eliminating the

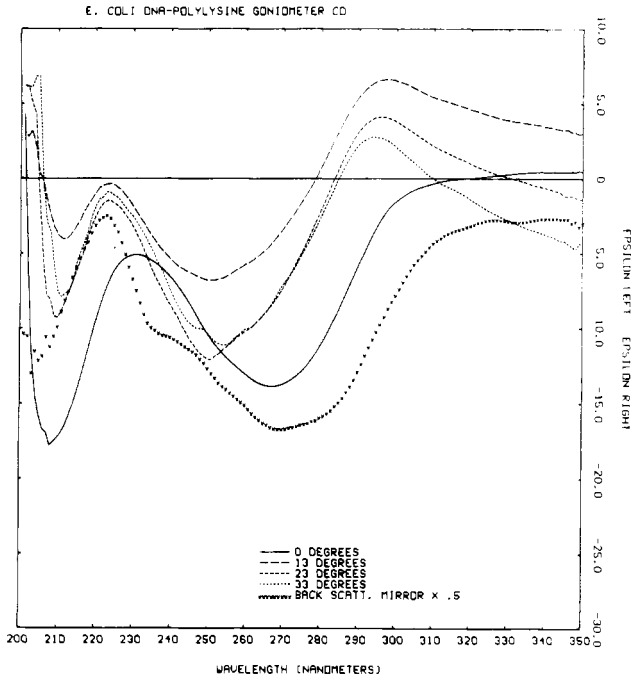

FIGURE 8: The *E. coli* DNA–polylysine particles of Figure 9 were measured by the goniometer device (Figure 1a) to determine the characteristics of the CD spectrum scattering. A comparison with film scattering (Figure 5) shows very similar behavior qualitatively, although the magnitudes are smaller in the particle case. (×) Backscattering obtained with the mirror device shown in Figure 1d.

CD tails in the region between 270 and 290 nm. However, at the same time it increases the magnitude of the minimum from $\Delta\epsilon$ (249 nm) = −3.6 to $\Delta\epsilon$ = −5.0. This is an analogous enhancement of the signal similar to the values reported in the EtOH–DNA particle study preceding this report.

For another type of particle, *E. coli* DNA–polylysine particle (unpublished results), its CD is essentially unchanged by the FDCD correction. It is evident from those particle studies that only a portion of the CD scattering pattern is "correctable" by large acceptance angle methods and that for those particles as well as the EtOH–DNA particles (Reich et al., 1980) a certain type of signal is not correctable and is even enhanced by increasing the collection efficiency of the detection system.

The CD scattering as a function of scattering angle θ (Figure 8) shows remarkably similar behavior to that of the DNA films (Figure 5). The backscattering as measured by the mirror collection device (Figure 1d) again shows the qualitative characteristics of the forward CD scattering or "normal" CD measurement.

The CD scattering behavior of *E. coli* DNA–EtOH particles had extremely large CD values, i.e., $\Delta\epsilon$ (normal, 275 nm) = +80. For such a large "resonance" CD, the FDCD methods enhanced the CD at the maximum up to a value of $\Delta\epsilon$ = +120.

One of the characteristics of the FDCD method is that the efficiency of correction increases as the total optical density of the solution is reduced. A very low concentration of the DNA–EtOH particle solution was measured with a concomitant increase in noise in the signal. The magnitude of the signal did not change radically, so that the CD spectrum at the resonance band is not a simple differential scattering signal but probably a manifestation of a liquid crystal (cholesteric or nematic) resonance reflection band having the associated anomalous transmission phenomenon.

Discussion

We propose the thesis that the optical activity of the films, the DNA particles, and the polylysine–DNA aggregates is a manifestation of a type of long-range chiral asymmetry similar in nature to that found in cholesteric liquid crystals and twisted-nematic liquid crystals. The evidence for the above assertion is as follows:

(a) Very large circular dichroism and ellipticities are shown. In the films the circular dichroism ranges from $\epsilon_l - \epsilon_r$ = +800 to −220, two orders of magnitude larger than the intrinsic magnitude of DNA solution. For DNA ethanolic condensates, we have shown values as large as +150. For DNA–histone complexes, values of the order of $\Delta\epsilon$ = −80 have been measured (B. P. Dorman, M. F. Maestre, and G. D. Fasman, unpublished results).

(b) The sense of the measured optical activity can be directly correlated with the sense of the twist of the film. Moreover, for any given film this is a reversible phenomena; i.e., if the sense of the twist is reversed, so is the sign of the measured CD bands. Thus in Figure 1 we show that a film with a counterclockwise twist of the top quartz plate, which produces a right-handed helix, will produce large positive ellipticities at wavelengths λ_1 = 298 nm and λ_2 = 233 nm. When this film is twisted in a reversed (clockwise) motion inducing a *left*-handed helical twist or distortion, we observe large negative values with minima at λ_3 = 280 nm and λ_4 = 210 nm. This reversal of ellipticity can be produced several times until the orientation of the film is destroyed by the continual manipulation of the quartz plate.

(c) The position of the CD maxima and minima of the films seems to be related to their degree of hydration or thickness. As the films dry out or are squeezed mechanically so that they become thinner, the positions of the bands shifts toward the blue, i.e., smaller wavelength. Conversely, when the films are hydrated again, the wavelengths of the bands increase in value (red shift). This particular property mimics that found in cholesteric crystals in which a Bragg law behavior is seen at those wavelengths of circularly polarized light which match the periodicity and sense of helicity of the liquid crystal. For cholesterics or twisted nematics (Maugin limit), we have

$$n\lambda = 2L \cos r' \qquad (8)$$

where n = order, λ = wavelength at which the anomalous reflection or transmission occurs, L = periodicity of helix, and r' = the internal refractive angle (obtained from Snell's law). Thus for a beam at perpendicular incidence and n = 1 (first-order reflection), we conclude that at wavelength $\lambda = 2L$ we should measure a very strong circular dichroism. However, in most of the CD spectra reported here we measure two bands. Even in the case where there is only a positive band, as in the DNA ethanolic particles, we see evidence of another band in the shorter region of the spectrum. It is probable that these two bands are a manifestation of two different modes of interaction of a large order helix with light. Furthermore, these bands show certain spatial distributions which also imply this interpretation, as we shall discuss below.

(d) The DNA films also show a remarkable behavior as a function of angle of incidence of the light beam. As shown in Figure 6, as the film is tilted with respect to the light beam incidence, we go through a minimum angle at ±20° from the vertical in which the first negative peak at λ = 270 nm disappears. As the angle is increased, we get a recovery of the CD peak. This is the kind of optical behavior that is seen in cholesteric liquid crystals with a well-defined structure and is interpreted as a shift in critical wavelength outside of the absorbance region produced by the Bragg law behavior.

The scattering behavior of the films, the DNA–EtOH particles, and the nucleohistone–DNA and polylysine–DNA particles show that the measured CD is mainly a scattering

resonance phenomenon that occurs at certain critical wavelengths. In particular the measurement of the backscattering regions of the film show magnitudes of the same order as those measured in the forward direction. At the critical wavelengths, the circular polarization of the scattering seems to remain constant, as a function of scattering angle. However, in the region of the spectrum away from the critical wavelength we observe a reversal of the sense of circular polarization. Thus, different regions of the CD spectrum of these structures behave with a different spatial distribution which indicates that they are produced by different scattering mechanisms.

(e) What is most remarkable is the large increase in the magnitude of some of the CD curves at the critical wavelength when attempts are made to recover all of the scattered light by large-angle integrating methods such as FDCD or fluorescent cells. Thus when the scattered light is measured over a sphere of 4π steradians, there is a sudden increase in the measured radiation of one polarization at a critical wavelength matching the absorbance bands of the DNA molecule. This is equivalent to an increase of transmission at a specific wavelength for light of one polarization over the other. A macroscopic model has been described by Chandrasekhar (1977) in terms of a thin film of cholesteric crystals with absorbing chromophores in the region of the spectrum with wavelengths that match the periodicity of the liquid crystal. It was shown by the model that the complex part of the index of refraction is the factor that influences the transmission of light reflected, while the transmitted polarization remains essentially unchanged since it does not interact differentially with the helix. Experiments conducted by Suresh (1976) and Nitayananda et al. (1973) confirm the prediction of the model for the circular dichroism of cholesteric crystals whose reflection bands overlap strongly the absorption bands.

A model system of optical activity was studied by Tinoco & Freeman (1957) using helical coils of copper wire and a microwave generator. These workers found that the coils showed strong Cotton effects at wavelengths determined by the periodicity of the wire helix and given by the equation $n\lambda \simeq 2L$ (where L is a linear function of the repeat period of the wire coil). There were two circular dichroic bands, one at the axial mode of interaction of the coil ["end fire" mode in the antenna theory; see Krauss (1950)] and the other at the shorter wavelength at the normal mode of oscillation of the coil.

Figure 3 shows the backscattering cone as measured by FDCD, and Figure 4 shows it as measured by the mirror-back collection device. In both cases the higher wavelength CD peaks have the same polarization as the CD peak measured in the forward direction. The short-wavelength peak is either absent or suppressed in magnitude. We are led to the assumption that this mode is probably oscillating in a perpendicular direction to the light beam and thus is weakly detected by backscattering detection devices.

Therefore, because of reasons enumerated above, we have concluded that the extremely large CD values reported for films, nucleohistones, and certain types of condensed DNA at specific wavelengths reflect a certain long-range order of a helical nature. The sense of the helicity is reflected in the sign of the so-called ψ spectra (i.e., a positive CD reflects a right-handed helical arrangement, and a negative CD reflects a left-handed coil). Furthermore, the positions of these large peaks reflect the repeat periodicity and are probably given by a Bragg reflection law analogous to the law for cholesteric liquid crystals (eq 8) or to that obtained for helical antenna arrays (Krauss, 1950)

$$n\lambda = 2L \text{ (axial mode)}$$

$$n\lambda = 2d \text{ (normal mode)} \quad (9)$$

where L = helical periodicity and d = diameter of helix.

It is important to realize that it is the helical ordering that provides the mechanism for the large CD values. Any random packing of nucleic acids will not produce any change in CD except that produced by changes in secondary structure. Gosule & Schellman (1976) have reported DNA–putrescine and DNA–spermine aggregates of very defined morphological sizes in which no changes in CD were detected. Similarly, Tunis-Schneider & Maestre (1970) have shown that films prepared in the proper way show little difference from the CD of DNA and RNA. We have found that it is easy to change one of the ψ-type films to obtain the regular conservative CD spectra of DNA by the proper manipulation of the quartz plates.

It is also important to note that the secondary structure of the DNA has little or nothing to do with the ψ-type spectra. It may be possible to correct for the ψ spectra by tilting the films until a null angle is obtained and then measure the remaining spectra which are presumably caused by the secondary structure, but this is a difficult experimental problem. In particles in solution this will be possible only if the ψ-type bands are fortuitously far away from some of the significant CD spectrum regions of the DNA which reflect secondary structures (for example, the 270–290-nm region).

The last remaining topic of discussion concerns those CD scattering perturbations that can be corrected by integrating methods, e.g., the long tails outside of the absorbance regions of the spectrum. These tails are also measured in the CD spectrum of cholesteric crystals (Holzwarth & Holzwarth, 1973). Invariably the tails can be corrected by FDCD or fluorscat cell methods indicating that the light is lost by differential scattering (i.e., one polarization is deviated more from the optical axis than the other). The explanation for this behavior is reasonably straightforward. It is a manifestation of the entire size and shape of the particle (i.e., Mie or "form" type of scattering), but in this case differential scattering occurs for different polarizations. This is also true for bacteriophage T2 and T4 (Dorman & Maestre, 1973; Holzwarth et al., 1974). These tails were shown to be proportional to the Kronig–Kramers transforms of the fluorscat corrected signal of the ψ-type spectrum. Therefore, in this sense it is a true Mie-type optically active or "form" dichroic scattering phenomenon. The DNA–EtOH particles show a more complicated behavior since for this type of particle the corrected scattering is at right angles to the beam. This is probably a reflection of the smaller size of the particle obtained for this particular set of measurements. We did not obtain a large backscattering reflection with FDCD and the mirror device for these particles; thus most of the scattering components are probably perpendicular to the light beam. Therefore this type of "correctable" CD scattering is a reflection of index of refraction dispersion and the bulk size, shape, and sense of helicity of the particle.

The nature of this type of scattering is analogous to that of form birefringence manifested by arrays of rods or plates when studied with linearly polarized light (Wiener, 1926). In this case, the form effect is a *circular birefringence form effect* which differentiates between right-handed and left-handed helical structure.

It should be noted that in the case of the polylysine T7 DNA condensates (Figure 8), there is a strong backscattering component; therefore the resonance mode of liquid-crystal-type scattering points to a different organization than that of DNA–EtOH condensates.

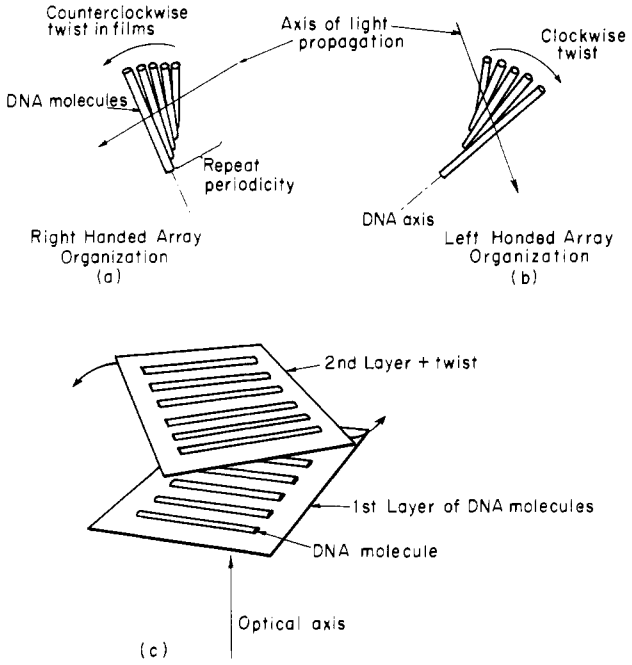

FIGURE 9: Proposed model of DNA packing in films and aggregates. (a, b) Sense imposed upon the film by the mechanical twisting; (c) actual interpretation given to twisted-nematic and cholesteric liquid crystals applied to the DNA fibers in a twisted DNA film.

Conclusion

From the studies of the CD and the scattering differential CD of films of DNA with twisted helical structure, polylysine–DNA ψ-type particles (negative), and DNA–EtOH salt aggregates (positive ψ spectra), we have arrived at the following conclusions.

(a) ψ-type CD spectra are mainly a manifestation of resonance-scattering phenomena with an associated anomalous transmission in the absorbing bands.

(b) The CD bands outside of the absorbing region can be corrected by the use of devices that increase the collection geometry such as fluorscat cells and FDCD methods. This is the birefringent dispersion component and is not only a function of the bulk index of refraction but reflects the size and shape of the particle as pointed out by Holzwarth & Holzwarth (1973) and Holzwarth et al. (1974).

(c) We have shown that the CD scattering of these particles can be divided into two types which reflect the different properties of the scattering structure. Information can be obtained on the long-range organization by demonstrating that some of the scattering exhibits optically active behavior such as is found in cholesteric crystals or twisted-nematic crystals. In such cases the sense of twist or superhelix can be determined from the sign of the CD bands. A right-handed helix gives positive CD signals and a left-handed helix gives negative CD signals. The periodicity is given by the Bragg law: $n\lambda = 2L \cos r$. Thus for a typical $\psi(-)$ nucleohistone aggregate which exhibits a critical λ = 270–280 nm, the helical periodicities would be on the order of 1350–1400 Å. As indicated in the experimental results, it is probable that DNA molecules stacked side by side and dehydrated will twist from a parallel arrangement to a left-twisted arrangement as they dehydrate and come closer together.

(d) In the few cases in which correction can be obtained at low salt concentration for DNA–polylysine complexes, we find that the corrected CD spectrum has a decreased band in the 270–290-nm region, indicating that at this salt concentration the secondary structure of the DNA is changing toward the C-form geometry. However, since the resonance bands increase in magnitude, it is impossible to obtain any information concerning the secondary structure of these molecules. It would be possible to measure such information if some type of quasi-liquid-crystal bulk organization (such as a film) could be obtained and then measurement could be performed at certain null angles given by the Bragg reflection law for liquid crystals.

(e) A model of the geometry of DNA in these aggregates is shown in Figure 9. By a helical array we mean a parallel organization of DNA molecules which are then twisted slightly so that each layer of DNA molecules is at a slight angle of twist with respect to the two neighboring ones. Each layer of DNA would be then twisted with respect to the two neighboring layers in a manner similar to that found in cholesteric liquid crystals or twisted-nematic liquid crystals. The long-range order of these layers would then provide the necessary helical periodicity to produce the strong resonance bands measured by circular dichroism. The planes in the figure are placed far apart for clarity, but in the DNA film we assume that these planes are in proximal contact. Indeed from our experiment this contact seems to be so close as to bias the twist in only one direction since DNA films tend to give negative ψ-type spectra if not forced into a given twist mechanically or forcibly disoriented. Therefore it is probable that some type of minimum energy configuration exists between quasi-parallel arrangements of DNA molecules that imposes a long-range left-handed organization as in Figure 9b.

(f) The above would explain the behavior of condensed DNA in bacteriophage T2, T4, and T6 in which an apparent liquid-crystalline component negative in value and centered at 260 nm is obtained (Holzwarth et al., 1974). The periodicity for the condensed T2 DNA package then would be of the order of 1300 Å if we assume that it obeys the Bragg scattering law, eq 8 for a first-order reflection. The CD tails would also be a property of the size of the particle since they are caused by birefringence dispersion. Smaller bacteriophages such as T5 and T7 (Maestre et al., 1971) show a much smaller contribution to the CD tails.

Supplementary Material Available

An appendix giving the equations for DNA film scattering (5 pages). Ordering information is given on any current masthead page.

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Constrained Configuration of Double-Stranded Ribonucleic Acid in HeLa hnRNP and Its Relaxation by Ribonuclease D†

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ABSTRACT: Double-stranded RNA (dsRNA) sequences within hnRNP from HeLa cells have been probed by means of ethidium bromide binding as monitored by fluorescence enhancement. About 8% of total RNA present in these particles was able to intercalate ethidium bromide and therefore appeared to be double stranded. Isotherm binding plots were markedly biphasic, the first dye molecules being bound with an affinity constant ~100 times higher than that of authentic dsRNA. Evidence for anticooperative binding was obtained by comparing the data obtained with ethidium bromide and with another intercalating drug, 2,6-dimethylellipticinium acetate, whose unwinding angle is half that of ethidium. This anticooperative effect can be explained by the existence in native hnRNP of a constrained configuration of dsRNA sequences. This interpretation is supported by a very high polarization of ethidium bromide fluorescence. This highly rigid structure is maintained through interaction with hnRNP proteins as treatment with proteinase K completely abolishes the anticooperative effect. Furthermore, we have shown that a dsRNA-specific RNase (RNase D) purified from Krebs ascites cells [Rech, J., Cathala, G., & Jeanteur, Ph. (1976) *Nucleic Acids Res. 3*, 2055–2065] was able to relax the constraint without appreciably reducing the absolute amount of dsRNA sequences. Treatment of hnRNP by a combination of RNases A + T_1 resulted in the complete disappearance of ethidium binding, confirming our recent report on the presence in hnRNP of an RNase D in the masked state which becomes active only after removal of endogenous RNA [Rech, J., Brunel, C., & Jeanteur, Ph. (1979) *Biochim. Biophys. Res. Commun. 88*, 422–427].

Among the various modifications suffered by hnRNA[1] when being processed into mature mRNA, the most drastic one is certainly the removal of extensive sequences through a splicing mechanism (Breathnach et al., 1977; Tilghman et al., 1978a,b).

The enzymology of splicing involves to start with a cleaving step which must be exquisitely specific at least when it occurs within coding regions (Breathnach et al., 1977; Tilghman et al., 1978a). Among the variety of structural features in RNA which a specific nicking enzyme might possibly recognize, we have elected to focus our attention on dsRNA regions. The rationale underlying this approach is fourfold. First, a precedent exists in prokaryotic systems in which RNase III which exhibits in vitro specificity toward dsRNA (Robertson et al., 1968) has been unambiguously involved in the specific cleavage of primary transcripts of *Escherichia coli* rRNA (Dunn & Studier, 1973; Nikolaev et al., 1973) as well as of T7 mRNA (Dunn & Studier, 1973). Second, dsRNA regions which exist in hnRNA (Montagnier, 1968; Stern & Friedman, 1970; Harel & Montagnier, 1971; de Maeyer et al., 1971; Jelinek & Darnell, 1972; Kronenberg & Humphreys, 1972; Ryskov et al., 1972; Patnaik & Taylor, 1973; Jelinek et al., 1974; Monckton & Naora, 1974; Torelli et al., 1974; Robertson et al., 1977) are mostly absent from poly(A)-containing cytoplasmic mRNA (Jelinek & Darnell, 1972). Third, there is sequence complementarity between mRNA and hnRNA (Stampfer et al., 1972) whose double-stranded regions can form duplexes with mRNA (Naora & Whitelam, 1975; Ryskov et al., 1976a,b). This suggests that only one of the two strands of some double-stranded regions is lost during processing and therefore that important cleavage sites may

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[1] Abbreviations used: hnRNA, heterogeneous nuclear RNA; hnRNP, ribonucleoprotein particles containing hnRNA; mRNA, messenger RNA; dsRNA, double-stranded RNA; snRNA, small nuclear RNA; EB, ethidium bromide; DMEA, 2,6-dimethylellipticinium acetate.

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