ABSTRACT

DNA films with $\psi^\pm$ CD spectra have been investigated. X-ray analysis has shown the sign of the $\psi$ spectra to be independent of the secondary structure of DNA. The appearance of the $\psi$ spectra is attended by the formation of a characteristic polygonal texture of the cholesteric type in the DNA film.

INTRODUCTION

CD studies of nucleic acids in neutral polymer solutions (1-3), in complexes with H1 histone (4,5), model polypeptides (6,7), polyamines (8,9), protamines (10), within mononucleosomes (11,12), and in DNA films at a low ambient relative humidity (13,14) have revealed abnormally intensive CD spectra. The intensity of these so-called $\psi$ spectra exceeds by far the characteristic optical activity of nucleic acids. Either these spectra lie entirely in the positive range of $\Delta\varepsilon$ values ($\psi^+$) or they are entirely in the negative range of $\Delta\varepsilon$ ($\psi^-$). The nature of these spectra could not be explained in terms of the proper optical activity of the nucleic acids, so it was hypothesized that the $\psi$ spectra might be connected with the formation of various supramolecular structures: a highly ordered tertiary structure (11), a mesomorphic structure (14), differential scattering of left and right-circularly polarized light by nucleic acid aggregates (3). However none of these hypotheses has had a clear experimental confirmation. The objective of the present study was to find out the origin of the $\psi$ spectra in DNA films.
MATERIALS AND METHODS

We used preparations of T7 phage DNA extracted at our laboratory and calf thymus DNA (Sigma) (1.0 - 1.5 mg/ml) dialyzed against various salt concentrations, as well as DNA from chicken erythrocytes (Reanal) dissolved in 1 SSC or 1M CH₃COONH₄(100 mg/ml). To prepare unoriented films (whose CD spectra did not change with the rotation of films relative to the light beam), molecular weights of T7 and calf thymus DNAs were reduced to about 500,000 by means of sonifying at melting ice temperature. Molecular weight of chicken erythrocytes DNA was 3.7x10⁶ Dalton.

Preparation of films. Thin unoriented films on quartz glass were prepared for CD measurements as described earlier (13). The same procedure was used to prepare the films for parallel CD and X-ray measurements but in this case a thin collodion film mounted on a hollow metallic cylinder 12 mm in diameter served as the foundation. The weight ratio of DNA to collodion was about 50. No appreciable differences were observed in the CD behaviour of DNA films on glass and on collodion. To prepare the films for polarization microscopy, a concentrated DNA solution (100 mg/ml) was applied to a surface confined by a glass ring (0.1 mm high) and spread by the motion of another glass over the surface of the thick film. To achieve equilibrium the films were placed in a chamber with relative humidity control (13).

Optical measurements. CD spectra were recorded by a modified according to (13) CD 6001 accessory of the Cary-60 spectropolarimeter. For absorbancy measurements we used a sensitive millivoltmeter attached to the CD 6001 accessory and graduated according to DNA concentrations in solution. An MI-8 polarization microscope was used for visual observation and photographing of DNA films.

X-ray measurements. To establish the secondary structure of DNA in a film, the latter together with supporting collodion film was taken off the cylinder in a box with relative humidity control, condensed and placed in an X-ray diffraction camera with relative humidity control. The specimen-to-film distance was about 40 mm and was measured by applying calcite powder
to the DNA specimen. We used a rotating anode X-ray tube (RI-GAKU, RV 200 PL).

Saturated solutions of inorganic salts (15) were used for keeping DNA films under fixed relative humidity.

RESULTS

As shown in (14) the decrease of r.h. may cause \( \psi \) spectra to appear in DNA films. We have found that the appearance of \( \psi \) spectra of DNA films depends on a number of parameters: relative humidity, type and concentration of salt in the specimen, mode of drying, volume of DNA solution applied. Various combinations of these parameters have been shown to cause only two types of \( \psi \) spectra (Fig.1) whose type and amplitude remain unchanged for several days under fixed conditions.

Table 1 presents the conditions in which DNA films displayed either positive or negative \( \psi \) spectra. One can see that the choice of experimental conditions allows spectra of both

![FIGURE 1. Schematic presentation of \( \psi \) spectra with different signs (we show the mean values of the amplitudes experimentally observed).](image-url)
TABLE 1. Conditions for the existence of $\psi$ spectra of different signs in DNA films.

<table>
<thead>
<tr>
<th>r.h.</th>
<th>type and concentration</th>
<th>DNA concentration</th>
<th>mode of drying</th>
<th>volume of solution</th>
<th>sign of spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>84% and less</td>
<td>NaCl 5x10^{-4}M</td>
<td>$1.6 \mu g/ml$</td>
<td>fast</td>
<td>25 $\mu l$</td>
<td>$+$</td>
</tr>
<tr>
<td>84% and less</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>50 $\mu l$</td>
<td></td>
</tr>
<tr>
<td>90% and less</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>slow</td>
<td>25;50 $\mu l$</td>
<td></td>
</tr>
<tr>
<td>95%</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>slow</td>
<td>100 $\mu l$</td>
<td></td>
</tr>
<tr>
<td>90% and less</td>
<td>NaCl $10^{-2}M$</td>
<td>$1 - 1.5\mu g/ml$</td>
<td>fast &amp; slow</td>
<td>50 $\mu l$</td>
<td></td>
</tr>
<tr>
<td>90% and less</td>
<td>CH$_3$COONH$_4$ $10^{-2}M$</td>
<td>$1.35\mu g/ml$</td>
<td>fast &amp; slow</td>
<td>50 $\mu l$</td>
<td></td>
</tr>
<tr>
<td>84% and less</td>
<td>CH$_3$COONH$_4$ $10^{-2}M$</td>
<td>$1.1\mu g/ml$</td>
<td>fast</td>
<td>50 $\mu l$</td>
<td>$+$</td>
</tr>
<tr>
<td>90% and less</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>slow</td>
<td>50 $\mu l$</td>
<td></td>
</tr>
</tbody>
</table>

* "Fast drying" means immediate placing of wet film into low r.h. environment (84% or lower). "Slow drying" means step-wise transition from higher (98-95%) to lower r.h.

It was possible to obtain films with $\psi$ spectra of different signs in the conditions of existence of both A-form and B-form DNA. We have noticed that a temperature rise causes a decrease in the amplitude of the $\psi$ spectra while films of fully denatured DNA exhibit no $\psi$ spectra at all. The amplitude of the $\psi$ spectra also showed a tendency to decrease with increasing molecular weight of DNA.

**X-ray analysis of DNA films.** We used a method of our own to analyse the secondary structure of DNA in films displaying $\psi^+$ and $\psi^-$ spectra. We obtained ring X-ray photographs of unoriented DNA specimens (analogues of polycrystalline powder...
photographs) and compared these with averaged over rotation photographs of A-form and B-form DNA in oriented fibres (Figure 2). Analysis of the secondary structure of DNA in films with \( \psi^+ \) spectra has revealed, under different conditions, both the A conformation (low NaCl content, 75% r.h.) and the B conformation (low salt concentration, high r.h. or high salt concentration, low r.h.). Analysis of films with \( \psi^- \) spectra has also demonstrated the existence of both the A form and the B form. Hence it appears that there is no direct connection between the sign of the \( \psi \) spectra and the secondary structure of the DNA molecules in films. Moreover, the X-ray photographs of B-form DNA films with \( \psi^+ \) and \( \psi^- \) spectra often look quite similar, which means that the DNA

FIGURE 2. Powder-like X-ray photographs of unoriented A-form and B-form DNA specimens (a/ and c/ respectively) and X-ray photographs of oriented A-form and B-form DNA fibres (b/ and d/ respectively).
packing in a unit cell is the same in both cases. The same conclusion can be drawn for the A form as well. Hence our results demonstrate that the sign of $\psi$ spectra and types of lateral intermolecular interactions (which are different for A and B forms of DNA) are not connected. It has been reported in (9) that $\psi$ spectra of different signs are observed for different values of the mean distance between DNA molecules. Thus the data of (9) and ours taken together show that close intermolecular interaction is not a factor affecting the sign of the $\psi$ spectra.

Polarization microscopy of DNA films. The high intensity of the $\psi$ spectra (exceeding the intensity of normal DNA spectra by at least three orders of magnitude) and their dependence on the molecular weight and temperature call for a consideration of the hypothesized, mesomorphic nature of the $\psi$ spectra. We have carried out a number of experiments to test that hypothesis. First, when a concentrated DNA solution (100 mg/ml, gel) was applied to a glass surface with a confining ring 0.1 mm thick and a cover glass was placed over the gel with a slight shift, a polarization microscope showed a texture that looked like a nematic liquid crystal (Figure 3a).

FIGURE 3. Texture of a newly-prepared layer of concentrated DNA solution (nematic liquid crystal) - (a) and texture of the same specimen after several days in 98% r.h. (presumably cholesteric polygonal) - (b).
The preparation gets extinguished in crossed Polaroids when the direction of shift coincides with the direction of oscillations in the polarizer and the analyzer (i.e. after every 90°). The turntable's rotation in either direction from the extinction position results in clarification. Pressure upon the cover glass also causes clarification resulting from a re-orientation of the optical axis due to the mechanical deformation (typical behaviour of a nematic phase). Thus a newly-prepared thick film displays the properties of a uniaxial anisotropic fluid which are close to those of nematic liquid crystals. The film shows a weak dichroism which is quite accountable in terms of the optical activity of DNA.

After the film is left for several days with the cover glass removed in a chamber with 98% r.h. (to prevent drying up) it shows a different texture which resembles the polygonal cholesteric one (Figure 3b). Individual sections of the texture display a clear-cut birefringence but on the whole there is no identifiable optical axis. A shift again results in a nematic kind of texture. Films with the polygonal texture (Figure 3b) show $\psi^-$ spectra. The least intensive spectrum is presented in Figure 4 ($\Delta \varepsilon$ is only roughly estimated assuming that $c=100$ mg/ml and $t=0.1$ mm remain unchanged while the
film is kept in 98% r.h.). As a rule the spectra were more intensive so that they could not be fully recorded because of the instrumental limitations, and yet the intensity of the spectra in a thick film was at least one order of magnitude lower than in a thin film.

The polygonal texture was maintained in a film dried up to room humidity. Thus a thick liquid film with a free surface undergoes a textural rearrangement in time (subdivision of anisotropic regions) correlating with the appearance of the $\psi$ spectra, and both the texture and the $\psi$ spectra persist after the film dries up.

We also subjected dry (because of quick drying in the air) thin DNA films to polarization microscopy. Such films also showed a texture similar to the one in Figure 3b. The films that did not display the characteristic polygonal structure showed no $\psi$ spectra either. The differences in the intensity of the $\psi$ spectra for thin and thick DNA films may be due to a nonlinear dependence of the CD spectra on film thickness (in our case it changed by two orders of magnitude) and/or relatively high molecular weight of DNA preparation.

DISCUSSION

Our results as well as those reported by other authors suggest several conclusions.

1. The origin of the $\psi$ spectra is not connected with changes in the secondary structure of DNA molecules. Moreover, a direct comparison of the CD data and X-ray analysis for the same DNA films shows that there is no correlation between the sign of the spectrum and the secondary structure of DNA or between the sign of the spectra and type of packing of DNA molecules into unit cell.

2. In a certain range of concentrations DNA forms a mesomorphic structure (lyotropic liquid crystal). Desultory mentions of the fact may be found in earlier studies (16-18), but only recently systematic investigations of the mesomorphic properties of DNA and synthetic polynucleotides has been attempted (19, 20). It has shown the possibility of a nematic mesophase forming in concentrated solutions of DNA and synthe-
tic polyribonucleotides. On standing for several weeks the nematic mesophase of synthetic polyribonucleotides may transform into a cholesteric one. In our case too a nematic mesophase is formed which is then converted into a cholesteric-like mesophase under special conditions.

3. There are a number of reasons to believe that the appearance of the $\psi$ spectra is connected with the formation of the cholesteric structure:

a) The appearance of the $\psi$ spectra correlates with the subdivision of anisotropic regions in the concentrated DNA solution, a similar process to the nematic - cholesteric transition in liquid crystals; the $\psi$ spectra are only observed in films with a characteristic polygonal texture.

b) The intensity of the $\psi$ spectra of our DNA films exceeds by several orders of magnitude the proper optical activity of DNA molecules, which is characteristic of cholesteric liquid crystals.

c) The CD spectrum of a cholesteric liquid crystal may have the shape of the absorption spectra of its constituent molecules, which is exactly the case with the $\psi$ spectra.

d) Denatured DNA in a film does not show the $\psi$ spectra whereas rigid (which is necessary for a mesomorphic structure) double-stranded DNA molecules do show them.

e) The intensity of the $\psi$ spectra grows with decreasing molecular weight of DNA (which probably reflects the growing degree of orientational order of the mesomorphic structure as the DNA molecules get more rigid).

Further investigation of the mesomorphic structure of DNA may yield more detailed information about it which will in turn serve to reveal the general features of the supramolecular DNA structures in films and solutions that show anomalous $\psi$ spectra.

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REFERENCES