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Comparison of calculated and observed CD spectra of liquid crystalline dispersions formed from double-stranded DNA and from DNA complexes with coloured compounds

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The CD spectra of dispersions of DNA, in the form of cholesteric liquid crystalline droplets, in an aqueous continuum have been studied. Calculated curves have been fitted to experimental spectra. The amplitude and the sign of the intense absorption band of the purine and pyrimidine bases vary with the droplet size, the pitch and the twist sense of the cholesteric phase. The CD spectra of dispersions of the complex formed by DNA and a coloured intercalating antibiotic have been similarly studied. A general satisfactory level of fitting between observed and calculated CD spectra was found.

1. Introduction

The properties of liquid crystalline phases of nucleic acids have been extensively studied in a number of different countries [1–3]. From the results obtained by a number of workers a detailed picture has emerged. Double-stranded DNA forms a variety of lyotropic liquid crystalline phases (cholesteric, pre-cholesteric, nematic etc.). Furthermore the structures of these mesophases depend on the properties of the solvent and on the secondary structure of the DNA.

The study of bulk samples of DNA mesophases leads to the investigation of dispersions of liquid crystalline DNA droplets in an aqueous continuum. The properties of such dispersions are of interest for several reasons. From a theoretical point of view, because the physico-chemical properties of LC dispersions can differ considerably from those of the bulk phase. The main differences arise because of the 'size effect' [4]. This results from the contributions to the free energy of the dispersed particles which arise from the surface tension of the small droplets. There can also be packing defects within these particles caused by the curvature of the surface, and the structure within a droplet may not be the same as that in the bulk LC phase.

The study of LC dispersions of double-stranded DNA is interesting from the biological point of view since the physico-chemical properties of dispersions reflect some properties of these macromolecules in biological systems such as virus particles or chromosomes of primitive organisms.

Finally, from the practical point of view, LC dispersions are of interest because of their potential as sensitive elements for biosensor devices [5] intended for the detection of compounds interacting with DNA molecules.

The formation of DNA LC dispersions as a result of phase separation in polymer-containing solutions [5] is accompanied by the appearance of an intense (abnormal) negative band in the CD spectrum in the region of the DNA base absorption. The negative sign of the band arises from the formation of the left-handed helicoidal structure of the cholesteric phase from the right-handed DNA molecules [6]. The sign of the band can be switched to positive under certain conditions [7]. The physical reasons, which determine the amplitude and sign of abnormal optical activity for DNA LC dispersions have been inadequately investigated, despite a number of attempts to describe their optical properties [8–10]. Among the practically important, but unsolved problems, one can select the following:

(1) The influence of the mode of spatial distribution for the DNA LC dispersion particles on the amplitude of the intense band in the CD spectrum.

(2) The connection between the amplitude of the band in the CD spectrum and the particle size.

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(3) The connection between the amplitude of the band in the CD spectrum and the pitch of the cholesteric structure.

(4) The influence of additional chromophores inserted into the DNA structure on the physico-chemical properties of LC dispersions.

It is evident that answers to such questions concerning the abnormal optical properties of DNA LC dispersions require additional theoretical and experimental analysis. An approach to calculating the CD spectra of DNA LC dispersions in the framework of a model of a polycrystalline LC generalized for the case of cholesterics and LC dispersions with their own absorption bands has been undertaken in the present work. The results of these calculations are compared with experimental CD spectra of DNA LC dispersions.

2. Materials and methods

2.1. *Preparations and equipment used*

Low molecular mass ($0{\cdot}5$–$0{\cdot}7 \times 10^6$ Da) chicken blood DNA (Reanal, Hungary) was used after additional purification and depolymerization. Double-stranded synthetic polynucleotides poly(dA–dT)*poly(dA–dT) and poly(dA)*poly(dT) (Calbiochem, USA) were used without additional purification. Poly(ethyleneglycol) (PEG, molecular mass 4000, Ferak, Germany) was used without purification. A preparation of a synthetic anti-tumour compound of the anthraquinone group, mitoxantron (MX), was obtained from the Chemistry Department of Padua University (Italy) and used without purification.

The DNA, polynucleotide and MX concentrations in water–salt solutions were determined by measuring the optical density of these solutions using a Specord M40 spectrophotometer (Germany). The DNA LC dispersion and LC dispersions of [DNA–MX]-complexes were formed according to a procedure described previously [5]. The CD spectra of LC dispersions were taken using a 1 cm cell and a Jobin–Yvon, Mark III dichrograph (France).

2.2. *The theoretical calculations of circular dichroism spectra for liquid crystalline DNA dispersions*

This section describes the main principles of the theory of the optical properties of imperfectly absorbing cholesteric LC [11–13], which was used for description of the CD spectra of DNA LC dispersions. Our description of the optical properties of the DNA LC dispersion takes into account the available microscopic information about these materials. In particular, experimental data [5, 7] enable us to treat the particles of dispersions formed from double-stranded linear DNA molecules of low molecular mass ($0{\cdot}5$–$0{\cdot}7 \times 10^6$ Da) as spheres with diameter D for which, due to the high elastic constants of the cholesteric structure stemming from the rigidity of the secondary DNA structure, the liquid crystalline ordering is relatively undistorted [14]. In addition, the DNA LC dispersions are considered as polycrystalline systems with random distribution and orientation of individual particles, possessing their own absorption in the UV region due to the presence of the nitrogenous bases in the DNA structure. It is also assumed that the separate liquid crystalline particles have small enough sizes to justify the application of the kinematic approximation of the theory of diffraction to describe the optical properties of individual particles. An approach based on such assumptions was previously applied to imperfect non-absorbing LCs [15]. Calculations of the optical properties of absorbing cholesteric LCs in the case of light propagation along the cholesteric axis have also been performed previously [16, 17].

It is known, that the modification of the effective dielectric constant of a medium (ε_0) due to light scattering is proportional to the forward scattering amplitude of an isolated scattering object measured at the frequency of the propagating light (ω). Therefore [15]:

$$\varepsilon_{\text{eff}} = \varepsilon_0 + \frac{4\pi c^2}{v_0 \omega^2} \bar{\Psi}(0) \qquad (1)$$

where v_0 is the volume of a single dispersion particle, and $\bar{\Psi}(0)$ is the forward scattering amplitude averaged over all orientations of the particles, i.e. over all possible directions of the reciprocal lattice vector $\boldsymbol{\tau}$.

Taking perturbation theory to the second order in the framework of the kinematic diffraction approach, it follows that the forward scattering amplitude $\Psi(0)$, non-averaged over all orientations of vector $\boldsymbol{\tau}$, for light of eigen polarization ($\mathbf{e}_\tau$) [12], which is diffracted by the helical structure, for the case of spherical crystallites with diameter D, may be expressed as [15]:

$$\Psi(0) = \frac{[(\operatorname{Re}\delta)^2 - (\operatorname{Im}\delta)^2 + 2\mathrm{i}\operatorname{Re}\delta\operatorname{Im}\delta] \times [\mathbf{x}_0^2\boldsymbol{\tau}^2 + (\mathbf{x}_0\boldsymbol{\tau})^2](\mathbf{x}_0\boldsymbol{\tau})^2}{64\alpha^4\mathbf{x}_0^5\boldsymbol{\tau}^4\varepsilon_0^2\mathrm{i}} \times [1 + x^2/2 - \mathrm{i}x^3/3 - (1 + \mathrm{i}x)\exp(-\mathrm{i}x)] \qquad (2)$$

where $x = \alpha\mathbf{x}_0 D$, $\alpha = [\boldsymbol{\tau}^2 + 2(\mathbf{x}_0\boldsymbol{\tau})]/2\mathbf{x}_0^2$, and $\operatorname{Re}\delta$ and $\operatorname{Im}\delta$ are real and imaginary parts of the dielectric anisotropy of cholesterics, respectively.

After averaging $\Psi(0)$ over all possible orientations of the dispersed particles, the eigen polarizations can only be circular, because in a disordered dispersed system there is no preferential direction.

Carrying out the projecting polarization ($\mathbf{e}_\tau$) over the circular ones and averaging $\Psi(0)$ over all orientations of vectors ($\boldsymbol{\tau}$), one can obtain from the real and the imaginary parts of equations (1) and (2) the expressions (3 *a*, *b*), which describe the optical rotation of the plane

of polarization (ϕ/L) and the coefficients of transmittance ($I_{L,R}$) of waves with left-(L)- and right-(R)-circular polarizations, respectively:

$$\phi/L = \frac{3\mathbf{x}_0^2 D}{32\varepsilon_0^2}\int_{-1}^{1}\{[(\mathrm{Re}\,\delta)^2-(\mathrm{Im}\,\delta)^2] \times(\sin x - x\cos x - x^3/3) + 2\,\mathrm{Re}\,\delta\,\mathrm{Im}\,\delta(1+x^2/2-\cos x - x\sin x)\} \times(1+y^2)(y/x^4)\,\mathrm{d}y \quad (3a)$$

$$(I_{L,R})_\lambda = \exp\left[-\frac{3\mathbf{x}_0^2 DL}{16\varepsilon_0^2}\int_{-1}^{1}\{[(\mathrm{Im}\,\delta)^2-(\mathrm{Re}\,\delta)^2] \times(1+x^2/2-\cos x - x\sin x) + 2\,\mathrm{Re}\,\delta\,\mathrm{Im}\,\delta(\sin x - x\cos x - x^3/3)\} \times(1+y^2)(1\pm sy)^2 x^{-4}\,\mathrm{d}y\right] \quad (3b)$$

where $x = \tau D(y + \tau/2\mathbf{x}_0)$, τ is the reciprocal lattice vector which is equal to $4\pi/P$, and $\mathbf{x}_0$ is the wave vector of the incident radiation. L is the thickness of the 'effective' DNA layer determined from the formula $L = Cl/\rho$, where C and ρ are the concentration and the density ($\mathrm{g\,cm^{-3}}$) of DNA, respectively, and l is the thickness of the cell (1 cm). $\delta_\lambda = (\varepsilon_1 - \varepsilon_2)/2$ is the dielectric anisotropy of the cholesteric LC, $\varepsilon_1, \varepsilon_2 = \varepsilon_3$ are the principal values of the tensor of dielectric permittivity, and $s = \pm 1$, depending on the sense of twist of the cholesteric structure.

For practical application of equations (1–3) to describe the optical properties of the particles of the DNA dispersion, which possess an absorption band due to the presence of nitrogenous bases in the DNA or to intercalated coloured compounds ('external' chromophores), one can specify the dependence of the optical parameters of the DNA dispersions upon the wavelength of the light. For this purpose we assume that an individual DNA dispersion particle has a cholesteric structure with pitch P and a dielectric anisotropy δ, which contains resonant components for DNA as well as the absorption band of an 'external' chromophore molecule. For further consideration it is important that, as for the DNA, the 'external' chromophores have a local anisotropy of absorption, i.e. the anisotropy of the dielectric constant δ has an imaginary part for corresponding wavelengths. In the expression for dielectric anisotropy, it is convenient to specify the component, originating from the absorption bands, i.e. to present δ in the form:

$$\delta = \bar{\delta} + \sum_i \delta_i$$

To take into account the absorption of the nitrogenous bases, as well as of external chromophores such as, in particular, coloured antibiotics, one can assume that one of the main values of ε_i and, hence, of δ_i has the resonant form:

$$\delta_i \sim \frac{r_i}{\omega_{0_i}^2 - \Delta - \omega^2 + i\gamma_i\omega}$$

where the components r_i are proportional to effects of the concentration of chromophores and the strength of the oscillator, and Δ is a factor required by the effect of polarization of the medium.

It is known that the circular dichroism is the difference in absorption for incident L- and R-circularly polarized light. Traditionally, it is the intensity of the transmitted light which is measured; this is related to the absorbance by the standard formula:

$$I = I_0 \times 10^{-A}$$

where I is the transmitted intensity, I_0 is the incident light intensity, and A is the absorbance.

The usual experimental circular dichrograph gives the following ratio:

$$(I_L - I_R)_\lambda/(I_L + I_R)_\lambda = \text{signal}$$

where $(I_L)_\lambda = (I_{0L})_\lambda \exp(-A_L)_\lambda$ and $(I_R)_\lambda = (I_{0R})_\lambda \exp(-A_R)_\lambda$, if the material under study obeys the Beer–Lambert law for each polarization.

Using equations (3) the CD spectra, i.e. the dependence of the measured ΔA or $(A_L - A_R)$ value upon λ, for the DNA LC dispersions, were theoretically calculated by the simple formula:

$$\Delta A_\lambda = (A_L - A_R)_\lambda = \log[(I_R)_\lambda/(I_L)_\lambda]$$

From expressions (3), it follows that for circular dichroism in the region of absorption bands which are located far away from the region of selective reflection for the cholesteric LC structure, theory recognizes that the qualitative behaviour of the circular dichroism [11] is the same as that considered earlier for cholesterics with propagation of light along their helicoidal axes [16, 17]. In particular, an anisotropy of absorption of molecules for linear polarizations of light in the case of cholesteric LC dispersions gives rise to circular dichroism; the sign of the dichroism depends on the sense of the cholesteric helix and the position of bands of absorption about a region of selective reflection. The sign of circular dichroism is reversed with a change of each of the mentioned parameters. The sign of the band in the CD spectrum in the region of absorption of 'external' chromophores depends on their orientation with respect to the long axis of the DNA molecule. The presence of an 'effective field' results in a 'red' shift of the maximum of the absorption band and the CD spectra both for LCs and DNA LC dispersions in comparison with the absorption and CD spectra of isolated DNA molecules. The new qualitative result obtained for dispersions, in

comparison with other cases considered [16, 17] is the dependence of the amplitude of the band in the CD spectrum upon the size of the particles of the DNA LC dispersion, which follows from equations (3).

A comparison of the results of the calculations of the CD spectra for DNA LC dispersions with the experimental measured spectra permits us to adjust the values of some of the parameters of the theoretical model, so that the theory can satisfactorily describe the experimental results.

Calculations of the CD spectra for DNA LC dispersions by expressions $(I_{L,R})_\lambda$ using equations (3) were performed using an AT 386 computer. The parameters $\bar{\delta}$ and r_i which provide the best fit of the theoretical and experimental CD spectra, were used in the computational process.

3. Results and discussion

In this section we give a comparison of theoretical and experimental CD spectra for the DNA liquid crystalline dispersions.

Figure 1 (*b*) compares the CD spectrum of a water–salt solution of the initial DNA (curve 1) with the CD spectra for an LC dispersion (curve 2) and the LC (curve 3) formed from the same DNA in water–salt solutions containing PEG. The formation of DNA LC and of DNA LC dispersion is clearly accompanied by the appearance of an intense band in the CD spectrum in the region where the DNA bases absorb. The band in the CD spectra for both DNA LC and DNA LC dispersion has a shape identical to that of the DNA absorption, figure 1 (*a*), but the maximum of the band in the CD spectrum is indeed 'red' shifted ($\lambda \sim 270$–300 nm). Finally, the value of $\Delta\varepsilon_{270}$ (~ -80 units) which characterizes the optical activity of the nitrogenous bases in the structures of DNA LC and DNA LC dispersion is far larger than the value of $\Delta\varepsilon$ ($\sim 2{\cdot}5$ units) characteristic for the molecular optical activity of the nitrogenous bases in a solution of isolated linear DNA molecules.

The combination of these facts suggests that the purine and pyrimidine bases do play the role of chromophores providing the information about the cholesteric packing of DNA molecules in the LC bulk phase and in the particles of LC dispersions. We could argue against this statement, because the intense band in the CD spectrum appears in the UV region where optical effects due to light scattering may result in considerable distortion of the shape of the band in the CD spectrum [18, 19]. Therefore the question of the shape of the theoretical CD spectrum for DNA LC dispersions without a light scattering contribution is of interest.

Figure 2 represents the theoretical CD spectrum for a DNA LC dispersion. This CD spectrum has an intense band in the region of absorption of the nitrogen bases (chromophores) of DNA. Both this band, and the band in the experimental CD spectrum of DNA LC dispersion with a left-handed cholesteric twist (see figure 1 (*b*) curve 2) have a negative sign, while the shape of the theoretical spectrum is identical to that of the DNA absorption band.

The coincidence of shape of the CD spectra for DNA

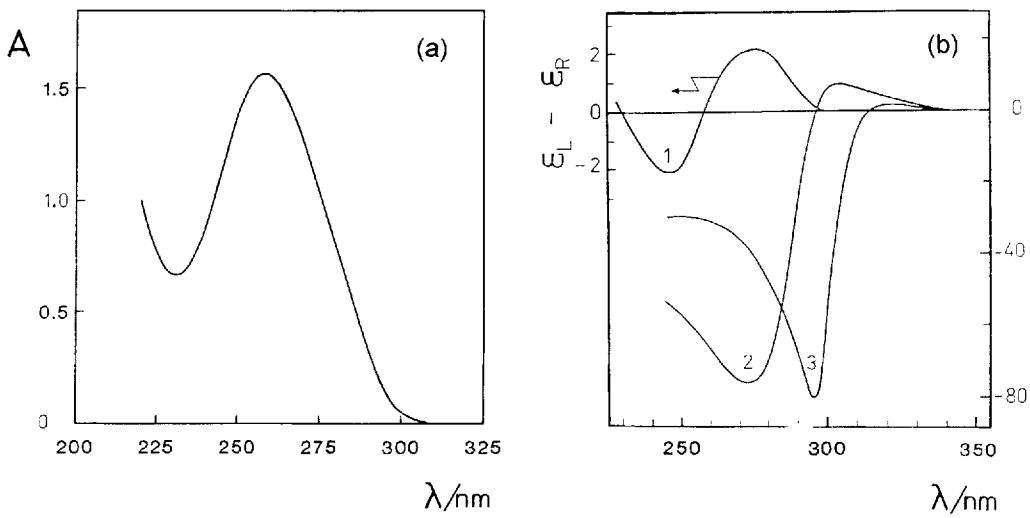

Figure 1. (*a*) The absorption spectrum of a water–salt solution of double-stranded linear DNA. DNA from chicken blood, Reanal, Hungary; mol mass $\sim (0{\cdot}5$–$0{\cdot}7) \times 10^6$ Da; 0·3 M NaCl + 10^{-2} M phosphate buffer, pH 6·7. (*b*) The CD spectra of water–salt solution of double-stranded linear B-form DNA (curve 1), of DNA LC dispersion (curve 2) and of a thin layer ($\sim 20\,\mu$m) of DNA LC formed in water–salt solution containing PEG: curve 1 left ordinate; curves 2 and 3 right ordinate. $C_{PEG} = 170\,\text{mg ml}^{-1}$; mol mass of PEG 4000 (Ferak, Germany); 0·3 M NaCl + 10^{-2} M phosphate buffer, pH 6·7.

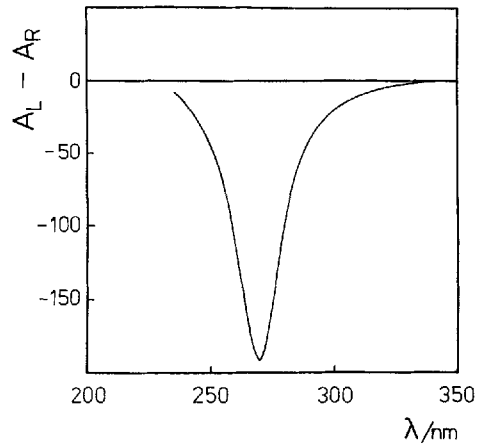

Figure 2. Theoretical CD spectrum for a DNA LC dispersion. $\Delta A = A_L - A_R$ in mm; 1 mm = $2{\cdot}5 \times 10^{-5}$ optical units. $C_{DNA} = 10\,\mu g\,ml^{-1}$; $L = 1$ cm.

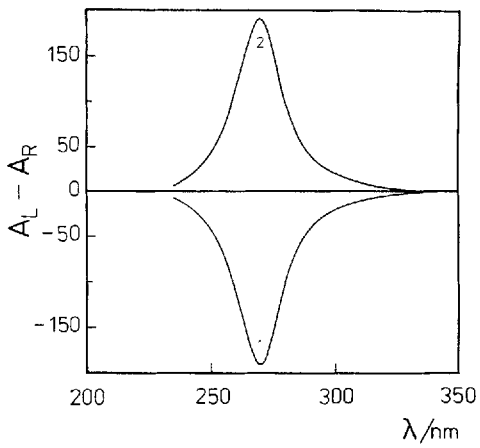

Figure 3. Theoretical CD spectra for DNA LC dispersions with left-handed (curve 1) and right-handed (curve 2) cholesteric twists. $\Delta A = A_L - A_R$ in mm; 1 mm = $2{\cdot}5 \times 10^{-5}$ optical units. $C_{DNA} = 10\,\mu g\,ml^{-1}$; $L = 1$ cm.

LC dispersion measured experimentally and the theoretical spectrum (figures 1 (*b*) and 2) shows that the method used to calculate the CD spectra of DNA LC dispersions, although phenomenological in its background, describes the optical properties of DNA LC dispersions properly. The experimental displacement of the maximum of the band in the CD spectra for DNA LC and DNA LC dispersion compared to the DNA absorption band, figure 1 (*a*), may also be connected with diffraction effects (together with the known polarization displacement due to 'effective field' effects acting on molecules in the cholesteric phase [20, 21]). This means that, together with the component of circular dichroism, which is determined by absorption ($\mathrm{Im}\,\delta_i$), there is a diffraction contribution to the circular dichroism, determined by the value of $\mathrm{Re}\,\delta_i$. In connection with different frequency dependencies of values of $\mathrm{Im}\,\delta_i$ and $\mathrm{Re}\,\delta_i$ near the absorption band (the value of $\mathrm{Im}\,\delta_i$ on resonant frequency reaches its maximum, but the value of $\mathrm{Re}\,\delta_i$ approaches zero at the main absorption band and has different signs on different sides of the main absorption band) the maximum of the band in the CD spectrum is shifted around the main band of absorption. The value of this displacement should, in principle, depend on the size of the particles of the DNA LC dispersion.

The role of different factors which can influence the amplitude and sign of the band in the CD spectra of DNA LC dispersions has been analysed.

Figure 3 compares the theoretical CD spectra for DNA LC dispersions with left-handed (curve 1) and right-handed (curve 2) cholesteric structures. The change in sense of the cholesteric packing of the DNA molecules in the LC dispersion results in a change of sign of the band from negative to positive. However, the shape of the CD spectra does not vary.

Figure 4 shows the experimental CD spectra of LC dispersions formed from right-handed synthetic double-stranded polynucleotides poly(dA–dT)*poly(dA–dT) (curve 2) and poly(dA)*poly(dT) (curve 4) with identical molecular mass, but different base sequences. These CD spectra are mirror-images of each other. In view of the experimental data [6], as well as of the results from the theoretical calculations (figure 3), the change of sign of the band in the CD spectrum shows that, in contrast to the left-handed cholesteric dispersions formed by molecules of poly(dA–dT)*poly(dA–dT), molecules of poly(dA)*poly(dT) form LC dispersions, with a right-handed twist. The CD spectra (figure 4) show that very small alterations in the structures of double-stranded molecules of nucleic acids can be sufficient to cause the change from a left-handed to a right-handed twist in the particles of the LC dispersions.

Figure 5 demonstrates the theoretical CD spectra for DNA LC dispersion particles of different size (at a constant pitch of the cholesteric helix equal to 2000 nm [2, 3, 5–7]). The amplitude of the negative band in the CD spectrum depends on the diameter of the LC dispersion particles and increases with its growth. The important conclusion, following from the data obtained, is that a size effect [4] is apparent. The theoretical calculation shows that if the diameter of DNA LC dispersion particles reaches ~50 nm, the amplitude of the intense band in the CD spectrum decreases so strongly, that it no longer differs from that in the CD spectrum characteristic of isolated linear DNA molecules in water–salt solutions, see figure 1 (*b*) curve 1. This result, in good agreement with earlier work [10], means that in the case of the formation of LC dispersion particles with a diameter ~50 nm, their presence cannot be registered by CD spectroscopy.

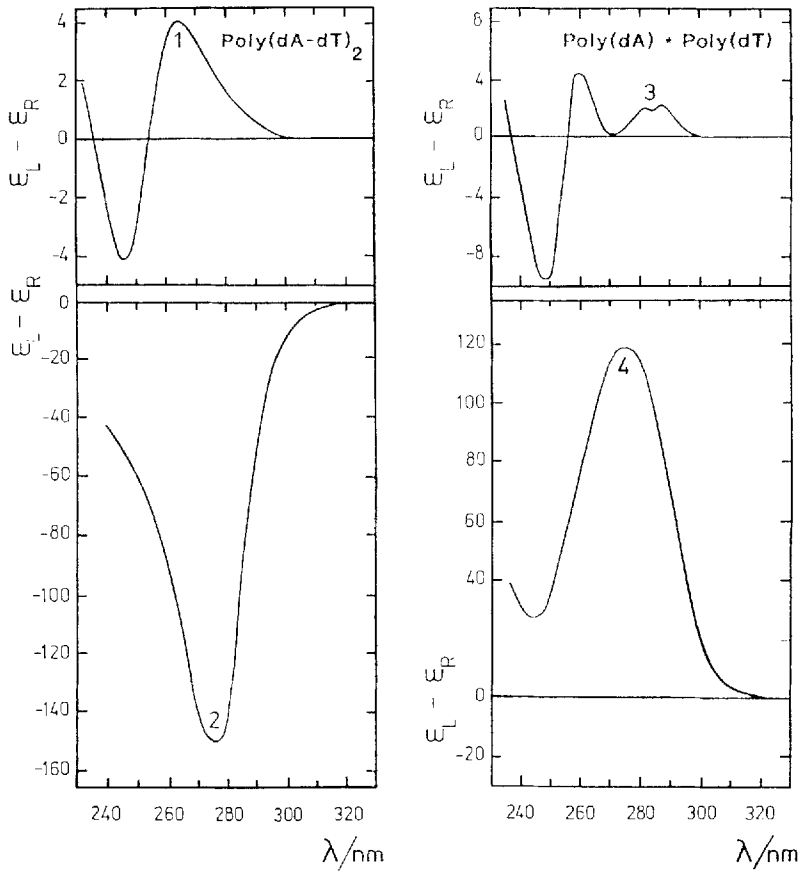

Figure 4. The CD spectra of water–salt solutions of double-stranded linear right-handed synthetic polynucleotides poly(dA–dT)*poly(dA–dT) (curve 1), poly(dA)*poly(dT) (curve 3) and their LC dispersions (curves 2 and 4, respectively) formed in PEG-containing water–salt solution. $C_{PEG} = 180\,mg\,ml^{-1}$; 0·3 M NaCl + 10^{-2} M phosphate buffer, pH 6·7.

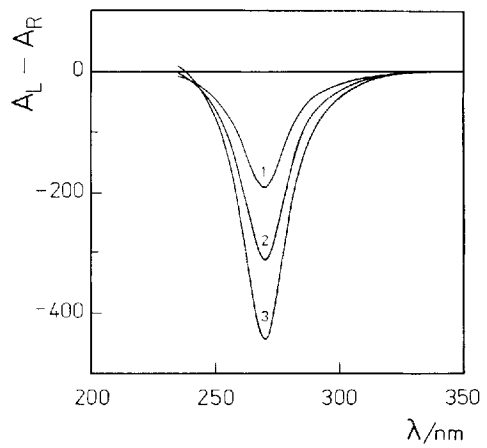

Figure 5. Theoretical CD spectra for DNA LC dispersions differing in their particle size. Curves 1, 2 and 3 correspond to particle diameters of 300, 400 and 500 nm, respectively. Pitch of cholesteric structure is 2000 nm. $\Delta A = A_L - A_R$ in mm; 1 mm = $2{\cdot}5 \times 10^{-5}$ optical units; $C_{DNA} = 10\,\mu g\,ml^{-1}$; $l = 1$ cm.

In the case of DNA LC dispersion particles with $D = 300$ nm, the influence of pitch of the cholesteric helix on the amplitude of the band in the CD spectrum was established.

Figure 6 exemplifies the theoretical CD spectra for DNA LC dispersions possessing different cholesteric pitches. The smaller the pitch of a cholesteric structure, i.e. the greater the twist, the more intense is the band in the CD spectrum and, conversely, the more untwisted the cholesteric structure, the lower is the amplitude of the band in the CD spectrum of the LC dispersion. The theoretical treatment indicates that where the pitch of the cholesteric structure of a DNA LC dispersion is ~30 μm and with constant structural properties of the double-stranded DNA molecules, the amplitude of the negative band in the CD spectrum is already close enough to the amplitude of the band characteristic of isolated linear DNA molecules, see figure 1 (*b*) curve 1. The experimental data [22] show that the untwisting of the helicoidal structure of the DNA cholesteric occurs under the influence of biologically active compounds;

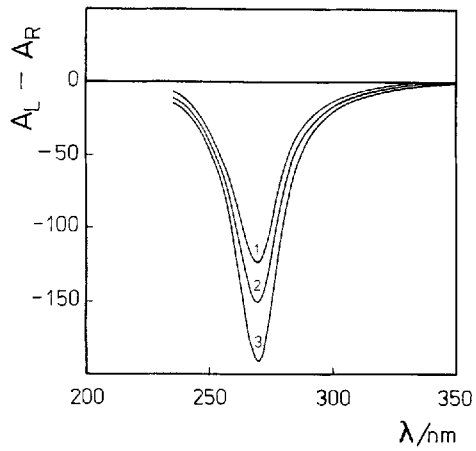

Figure 6. Theoretical CD spectra for DNA LC dispersions differing in the pitch of their cholesteric structures. Curves 1, 2 and 3 correspond to P values equal to 3400, 2700 and 2000 nm, respectively. Diameter of DNA LC dispersion particles is 300 nm. $\Delta A = A_L - A_R$ in mm; 1 mm = $2{\cdot}5 \times 10^{-5}$ optical units; $C_{DNA} = 10\,\mu g\,ml^{-1}$; $L = 1$ cm.

the amplitude of the negative band in the CD spectrum (at definite fixed concentrations of antibiotics) does not differ from that which is characteristic of linear DNA molecules already with a pitch ~8–10 μm. Attention is drawn to the fact that experimentally measured and theoretically calculated 'limited' P-values are of the same order of magnitude. Any difference between them may reflect the fact that during the experimental determination of 'limited' P-value, the secondary structural parameters of the DNA molecules can be varied by action of antibiotics.

The important question in analysis is the shape of the CD spectra for DNA LC dispersions with coloured compounds introduced into the structure of the DNA molecule. Figure 7, for example, shows the experimental CD spectra of LC dispersions formed from DNA complexes with the coloured anti-tumour compound, mitoxantrone (MX) which intercalates between the DNA bases. A few points attract attention. First, the CD spectra of the LC dispersions have two bands. One occurs in the absorption region of the DNA bases ($\lambda \sim 270$ nm) and the other lies in the absorption region of the MX chromophores ($\lambda \sim 680$ nm). At a low level of binding of MX with the DNA molecules, both bands have negative signs. Secondly, the shapes of the bands in the CD spectrum are identical to those in the individual absorption spectra of DNA and MX. Thirdly, the amplitudes of both bands (as calculated per mol of DNA bases per mol of MX bound to DNA) are similar. The identical signs of the bands in the CD spectra indicate that the orientation of the MX molecules with respect to the DNA molecular axis coincides with the orientation of the nitrogen bases.

Figure 8 shows the CD spectra calculated theoretically for LC dispersions formed from [DNA–MX] complexes. As well as in the experimental CD spectra (figure 7), the theoretical CD spectra contain two bands of identical sign in the regions of absorption of DNA and MX. This means, that the proposed method for calculation of the CD spectra predicts the appearance of an additional band in the region of absorption of the chromophoric moiety of the compound bound into the DNA structure. The best fit of theoretical and measured CD spectra corresponds to the following set of experimental parameters: $\bar{\delta} = -0{\cdot}14$, $r_{270} = 0{\cdot}098$. (Parameter r_{MX} charac-

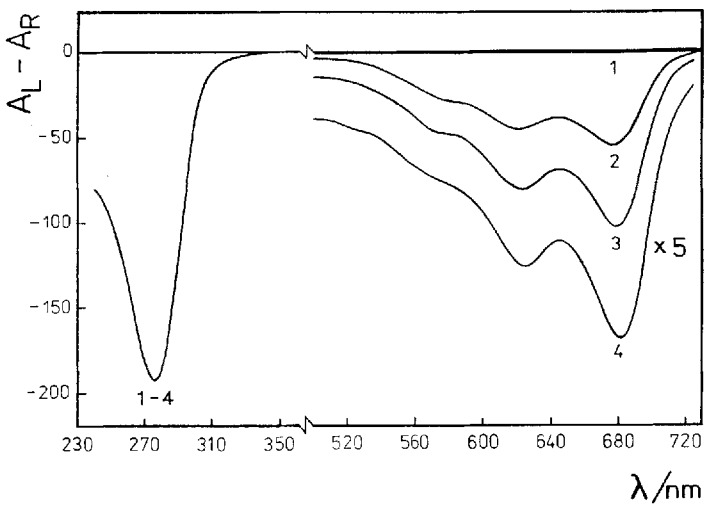

Figure 7. CD spectra for LC dispersions formed from [DNA–MX] complexes. Curve 1, $C_{tMX} = 0$; curve 2, $C_{tMX} = 1{\cdot}55 \times 10^{-6}$ M, curve 3, $C_{tMX} = 3{\cdot}08 \times 10^{-6}$ M, curve 4, $C_{tMX} = 5{\cdot}35 \times 10^{-6}$ M. C_{tMX} is the concentration of MX in solution. C_{PEG} = 170 mg ml^{-1}; 0·3 M NaCl + 10^{-2} M phosphate buffer, pH 6·7. $\Delta A = A_L - A_R$ in mm; 1 mm = $2{\cdot}5 \times 10^{-5}$ optical units; C_{DNA} = 10 μg ml^{-1}; $L = 1$ cm.

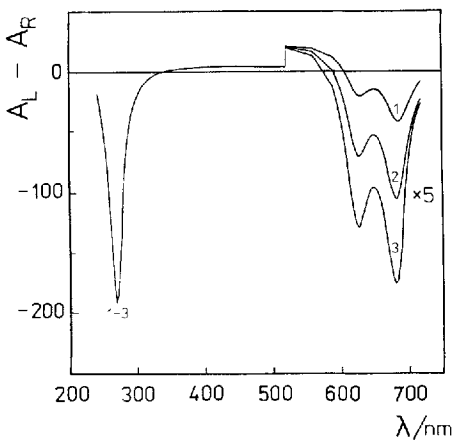

Figure 8. Theoretical CD spectra for LC dispersions formed from [DNA–MX] complexes. Curve 1, $r_{MX} = 0{\cdot}02$; curve 2, $r_{MX} = 0{\cdot}04$; curve 3, $r_{MX} = 0{\cdot}06$. Diameter of LC particles is 300 nm; pitch of cholesteric structure is 2000 nm. $\Delta A = A_L - A_R$ in mm; 1 mm = $2{\cdot}5 \times 10^{-5}$ optical units; $C_{DNA} = 10\,\mu g\,ml^{-1}$; $L = 1$ cm.

terizes the various MX concentrations; in the present case, r_{MX} values were chosen equal to 0·02, 0·04 and 0·06: the diameter of the LC dispersion particles was 300 nm, and the pitch of the cholesteric helix formed by the DNA molecules was 2000 nm.)

The comparison of the experimental and the theoretical CD spectra (figures 7, 8) shows that the amplitude of the band in the CD spectrum in the region of MX absorption grows as more MX molecules are bound to the DNA, although the amplitude of the band in the region of DNA absorption remains constant. It is possible to predict that such a situation will be observed so long as, under MX action, the parameters of the DNA secondary structure do not begin to vary or the intercalation mechanism of MX binding is not replaced by any other.

Thus, the approach developed in the present work for description of the CD spectra of DNA LC dispersions and its complexes can be used for a qualitative analysis of the influence of some LC dispersion parameters on their abnormal optical properties. The proposed approach allows us to evaluate such parameters as the relative orientation of the oscillators of absorption of the DNA bases and intercalated chromophores, to determine the characteristic size of individual LC dispersion particles, as well as to assign the sense of twist of the DNA cholesteric phase, etc. Outside the framework of the theoretical approach used in this work there remains, however, the question of the influence of solvent properties on the structural peculiarities of DNA.

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