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Invited Article Liquid-crystalline dispersions of nucleic acids

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Invited Article

Liquid-crystalline dispersions of nucleic acids

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The formation and properties of dispersions of double stranded natural and synthetic nucleic acids are described. Evidence is given for the liquid-crystalline state of the nucleic acid within the dispersed droplets in these phases. The exploitation of nucleic acid dispersions in biosensors is discussed.

1. Introduction

The properties of lyotropic liquid-crystalline phases of nucleic acids in water–salt, and in water–salt–polymer solutions, have been studied extensively by a number of workers (see, for example the review [1] or the paper [2]). Their observations suggest that only rigid, double stranded, optically active, low molecular mass (lower than 1×10^6) DNA molecules can form mesophases and that there is a sequence of mesophase structures: these include cholesteric, precholesteric and nematic. The specific structural features of DNA molecules as well as the properties of the solvent both influence the mesogenic properties of these systems.

The physical properties of dispersions often differ appreciably from those of the bulk phases. This is especially for super-dispersions where the particle size ranges from 100 to 1000 Å. This so-called dimensional effect [3] arises from the surface tension phenomena and from distortions of the bulk phase structure within the particles.

The physico-chemical properties of liquid-crystalline dispersions of nucleic acids are of biological interest [4, 5] because chromosomes and DNA containing viruses are isolated systems of microscopic size with an ordered but labile packing, despite the high rigidity of the DNA molecules and their high molecular mass. Finally, the properties of liquid-crystalline dispersions are of technological importance because they can be used in biosensors [6].

2. The properties of nucleic acid dispersions in water–salt–polymer solutions

Dispersions of nucleic acids are formed as the result of phase exclusion when their dilute solutions in a water–salt mixture are treated with some synthetic polymers, for example poly(ethylene glycol) (PEG) [1, 7]. The efficiency of phase exclusion is related to a number of variables (see figure 1). The formation of liquid-crystalline dispersions can be demonstrated using absorption spectroscopy. Starting from a particular PEG

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concentration in solution, the optical density in the absorption band of the nucleic acid nitrogen bases decreases (a so-called flattening of the absorption spectra [8]), whilst at wavelengths above 320 nm (where neither DNA nor PEG molecules absorb) it is increased (an apparent optical density, A_{app}, (see figure 1). Both optical effects indicate the formation of DNA dispersions. The diameter, D, of the particles formed has been calculated [9–11] using the relationship

$$A_{app} = K\lambda^n,$$

where λ is the wavelength and n is related under certain conditions to the diameter of the particles. For DNA dispersions, D is equal to about 10^3 Å. This tends to rise with DNA concentration and falls as the PEG concentration is increased [12]. For example, $D = 4{\cdot}5 \times 10^3$ Å at $C_{PEG} = 100\,mg\,ml^{-1}$ and $D = 3{\cdot}8 \times 10^3$ Å at $C_{PEG} = 300\,mg\,ml^{-1}$.

The size of the DNA particles is related to the molecular mass of PEG although the precise relationship has not been established [12].

The translational diffusion coefficient (D_T) has been found for DNA dispersions ($C_{PEG} = 170\,mg\,ml^{-1}$; PEG molecular mass = 4000; 0·3 M NaCl) using laser correlation spectroscopy: $D_T = 14 \times 10^{-10}\,cm^2\,s^{-1}$ as $C_{DNA} \to 0$. This value corresponds to spherical particles with $D = 3{\cdot}7 \times 10^3$ Å. The coefficient of sedimentation (S) has been determined for DNA dispersions by means of low speed centrifugation: $S = 14{\cdot}3 \times 10^3$ as $C_{DNA} \to 0$. The radius, R, of DNA particles has been calculated in terms of the Grosberg theory [13] that describes the condensation of the high molecular mass DNA molecules in polymer containing solutions. The theoretical value of R ($R = 2{\cdot}9 \times 10^3$ Å

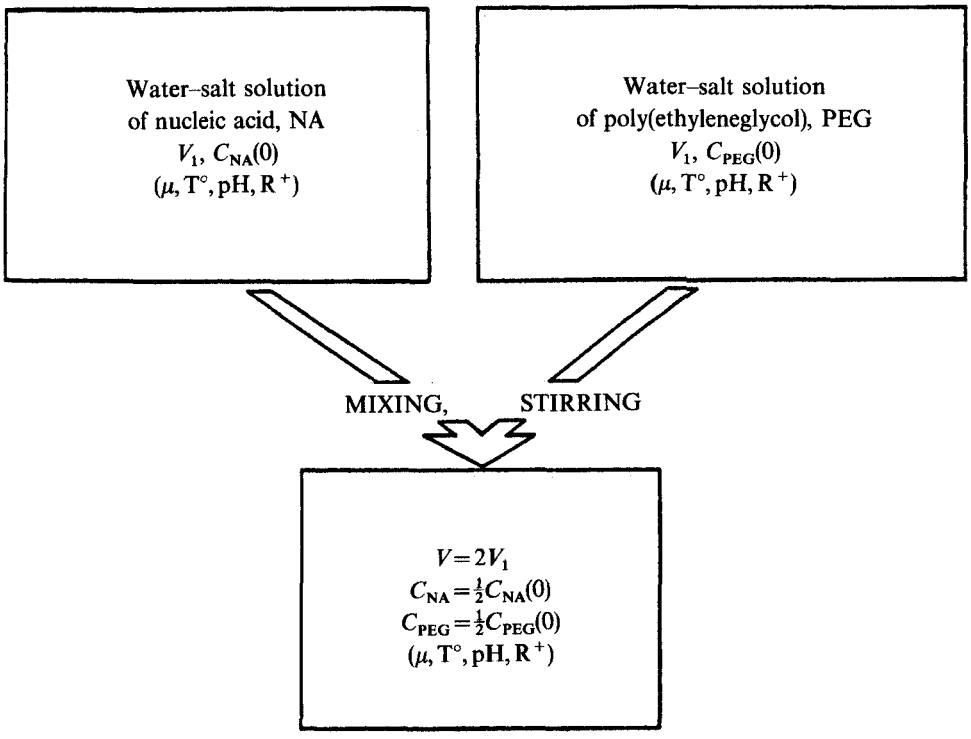

Figure 1. A general scheme for the preparation of dispersions of natural and synthetic nucleic acids.

at $C_{PEG} = 170\,mg\,ml^{-1}$) is the same order of magnitude as that found experimentally. Using the D_T and S values the molecular mass of a dispersed DNA particle is estimated as about 10^{10} [12]). Since the mean molecular mass of one DNA molecule is about 10^6, each particle of the DNA dispersion contains about 10^4 DNA molecules (at $C_{PEG} = 170\,mg\,ml^{-1}$). These results indicate that double stranded DNA molecules form dispersions in PEG containing solutions.

The formation of dispersions from molecules of other nucleic acids and synthetic polynucleotides in PEG containing solutions has also been examined [14, 15]. When water–salt solutions of PEG (molecular mass = 4000) were added to water–salt (0·3 M NaCl) solutions of double stranded RNA (the replicative form of phage f2, molecular mass about 2×10^6), an apparent optical density appears at $\lambda > 320\,nm$, just as for double stranded DNA, the amplitude of the intrinsic absorption band decreases, and its maximum shifts slightly to the longer wavelengths [10]. Similar changes in absorption spectra are observed for solutions of some double stranded synthetic polynucleotides: poly(I)xpoly(C), poly(A)xpoly(U), poly(dAT)xpoly(dAT) and poly(dA)xpoly(dT). These changes are less pronounced for denatured DNA or single stranded RNA. The absorption spectra of water–salt (0·3 M NaCl) solutions of poly(U) or poly(A) and poly(G), as well as of tRNA, in the presence of PEG (molecular mass = 20 000) do not demonstrate the formation of dispersions. Therefore, we can conclude that only rigid, double stranded molecules of nucleic acids can form separate particles (microphases) in PEG containing solutions. Both kinetic and thermodynamic factors appear to be responsible for the small size of the DNA particles in these dispersions [10].

Absorption spectra allow us to estimate a critical concentration of PEG (C^{cr}_{PEG}) at which the formation of DNA dispersions takes place (see figure 2) and to establish its dependence on the properties of the solution used. The determination of C^{cr}_{PEG} values for PEG containing solutions, where the molecular mass of PEG ranges from 400 to 40000, shows that the higher the PEG molecular mass, the lower the C^{cr}_{PEG} value. A DNA dispersion can be formed if the molecular mass of PEG exceeds 600.

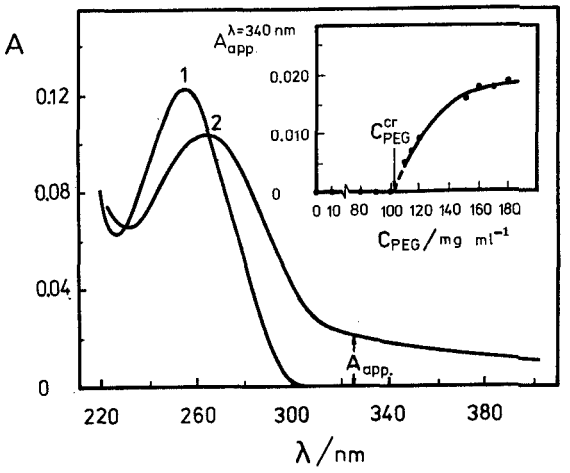

Figure 2. Changes in the shape of the absorption spectrum upon formation of the DNA dispersion in a water–salt (0·3 M NaCl)–PEG solution. Curve 1, absorption spectrum of the water–salt–DNA solution; curve 2, absorption spectrum 1 with $180\,mg\,ml^{-1}$ of PEG. Inset, changes in the apparent optical density (A_{app}) upon increase of the PEG concentration in a water–salt–DNA solution. The critical concentration of the PEG (C^{cr}_{PEG} value) is shown in the inset.

If the DNA molecule is regarded as a polyphosphate chain, we may explore some properties of water–salt solutions which influence their effectiveness in the formation of the DNA dispersions. When the ionic strength of PEG containing solutions of alkali metal salts is raised from 0·1 to 0·7, the C^{cr}_{PEG} values decrease; the minimal values correspond to sodium salts in all cases. If the anionic composition of PEG containing solutions is altered, this does not affect the C^{cr}_{PEG} values determined in a solution with a fixed cationic composition. The C^{cr}_{PEG} values decrease when passing from solutions of alkali metal to those of alkaline earth metal salts. For example, the ionic strength of a solution sufficient for the formation of a dispersion is 0·15 ($C_{PEG} = 170\,mg\,ml^{-1}$) for a Na^+ containing solution and only about 0·003 for a Mg^{2+} containing solution [16].

Thus, the conditions for microphase formation involve factors such as ionic strength, cationic composition of the solutions, as well as the concentration and molecular mass of PEG.

The data presented here questions the mode of packing of nucleic acid molecules in dispersions. Because of the microscopic size of the particles and their low concentration we can say that physico-chemical methods for determining a specific ordering of nucleic acid molecules inside the dispersions have yet to be developed.

3. Evidence of the liquid-crystalline state of nucleic acid particles in dispersions

The liquid-crystalline state of double stranded nucleic acid or synthetic polynucleotide particles in dispersions has been proved using the method of external chromophores [17]. This method is based on the observation that when molecules of coloured compounds are dissolved and oriented in thermotropic cholesteric phases of low molecular mass compounds they have an abnormal optical activity. This abnormal optical activity may be expressed, for instance, as an intense band in the circular dichroism (CD) or the optical rotation (OR) spectrum. DNA molecules have a helical structure and are optically active. In full agreement with theoretical predictions [18, 19] these molecules tend to form cholesteric phases. Hence, the first condition of this method can be realized. The second condition (namely the dissolution and orientation) is met in the sense that DNA molecules contain purine and pyrimidine bases. These bases absorb in the UV region of the spectrum and are fixed rather rigidly within the DNA helix. Their tilt angle with respect to the DNA helical axis is practically constant under any conditions as long as the DNA molecules are not denatured. Consequently, when the cholesteric DNA phases are formed, an intense band in the CD spectrum in the region of absorption of the purine and pyrimidine bases is expected. Obviously, such a band will be dependent on the pitch and will be absent in the untwisted nematic state. Hence, the optical properties can provide information about the state of the mesophases.

So, cholesteric liquid crystals of DNA are, in essence, dyed cholesterics. The intense band in the CD spectrum in the absorption region of the purine and pyrimidine bases is characteristic. Its amplitude is given by [20, 21]:

$$\Delta\varepsilon_{\nu_i} = kP\nu_i^3\Delta n(A_{\parallel} - A_{\perp})/(\nu_i^2 - \nu_0^2), \tag{1}$$

where $\Delta\varepsilon_{\nu_i}(\equiv\Delta\varepsilon_L - \Delta\varepsilon_R)$ is the circular dichroism at frequency ν_i; ν_0 is the frequency at which the selective reflection band of the cholesteric is observed; its value depends on the pitch (P) of the cholesteric helical structure ($P = k_1 n\nu_0$; P is positive for a right handed cholesteric superhelix). For lyotropic liquid crystals formed from DNA molecules (molecular mass lower than 1×10^6) P corresponds to several microns [1];

Δn is the optical anisotropy, this has a negative sign in our case; $A_{\parallel} - A_{\perp}$ is the linear dichroism of DNA, this depends on the angle of inclination (α) of the purine and pyrimidine bases with respect to the helix axis of DNA:

$$(A_{\parallel} - A_{\perp})/A = k_2(3\cos^2\alpha - 1); \tag{2}$$

the linear dichroism of the nitrogen bases has a negative sign. The band in the CD spectrum will have a sign depending both on the sense of the cholesteric twist and on the angle at which the bases are oriented with respect to the director. The negative sign of the CD band indicates the formation of a left handed cholesteric helix from right handed DNA molecules [21]. Since equation (1) does not clearly specify the size of a liquid-crystalline phase, the following idea suggested by the method of external chromophores was used in [20]: '...an intense band can be recorded in the CD spectrum in the region of absorption of the purine and pyrimidine bases not only for cholesteric liquid crystals but also for liquid-crystalline dispersions of nucleic acids if their molecular packing is similar to cholesteric liquid crystals'.

Figure 3 compares the CD spectrum for a water–salt solution of DNA with the CD spectrum for a dispersion formed by the same DNA in a PEG solution ($C_{PEG} > C^{cr}_{PEG}$). First, the CD spectrum has an intense band ($\lambda \approx 270$ nm) in the region where the bases absorb. Secondly, the band in the CD spectrum has a shape identical with that of a DNA absorption. Finally, the value of $\Delta\varepsilon_{270}$ (about 120 units) which characterizes the optical activity of the bases in the DNA dispersion is far larger than the value of $\Delta\varepsilon$ (about 2 units) characteristic for the molecular optical activity of the bases in isolated DNA molecules. The combination of these factors suggests that the purine and pyrimidine bases do play the role of external chromophores. This result permits us to suggest that cholesteric packing is typical of the DNA dispersions. We could argue against this suggestion because the CD spectrum has an intense band in the UV region where optical effects due to light scattering may distort the shape of the band in the CD

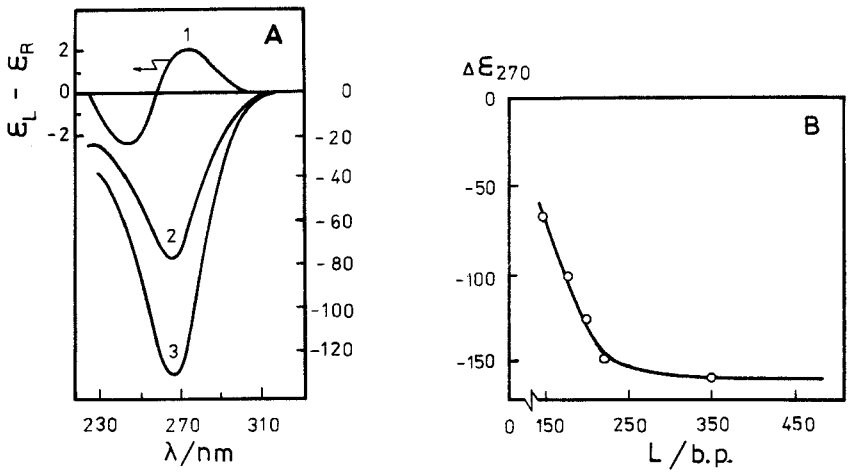

Figure 3. (A) The CD spectra of linear, double stranded, right handed helical B form DNA (curve 1, 0·3 M NaCl) and the dispersion phase formed in the water–salt–PEG solution (curves 2 and 3, molecular mass of PEG = 4000; $C_{PEG} = 170$ mg ml^{-1}, 0·3 M NaCl; the DNA molecular mass decreases when passing from preparation 2 to 3). (B) The amplitude of the negative band in the CD spectrum ($\Delta\varepsilon_{270}$) for the PEG containing solution against the DNA length (nucleosomal DNA from the chromatin of Ehrlich tumour ascite cells). DNA length (L) is given in base pairs (b.p.); 1 b.p. is 3·4 Å.

spectrum considerably [22, 23]. Therefore, it is necessary to analyse the role of various factors which might be affected by light scattering and which can in turn influence the amplitude of the band.

The amplitude of the band in a CD spectrum depends on the length of the DNA molecules. When they are short (less than 50 b.p. or about 150 Å) no dispersion with an abnormal optical activity can be formed (see figure 3). We may conclude, therefore, that very short molecules cannot condense in PEG containing solutions, that is there exists a lower limit of DNA molecular length for a dispersion to be formed. The amplitude of the band in the CD spectrum decreases as the DNA molecules become longer although light scattering persists in the UV spectral region. The molecular mass of DNA has an upper limit of 3×10^6 [24] at which these molecules are still able to form a dispersion with abnormal optical acitivity. When the molecular mass exceeds 10×10^6, the CD spectra of dispersions, despite the light-scattering, are very similar to those typical of uncondensed linear DNA molecules. The absence of any abnormal optical activity, for dispersions of high molecular mass DNAs indicates that long chain DNA tends not to form a cholesteric phase. The amplitude of the band in the CD spectrum depends on C_{PEG} in a complex manner (see figure 4). A negative band appears in the CD spectrum only after C^{cr}_{PEG} has been reached. Its amplitude reaches a maximum at $C_{PEG} \approx 160$–$190\,mg\,ml^{-1}$, and then drops abruptly.

The effectiveness of formation of DNA dispersions with an intense band in the CD spectrum depends on the nature of the cations and not on the nature of anions present in solution (see figure 4). The value of C^{cr}_{PEG} is lower by $60\,mg\,ml^{-1}$ in a solution with Na^+ than in a solution containing Cs^+ cations and having the same ionic strength. Nucleic acid dispersions with an abnormal optical activity are formed in PEG containing solutions only when 82 per cent of the negative charges of the phosphate groups are neutralized with counterions [16]. These data are consistent with theoretical and experimental estimates [25, 26] which characterize the neutralization level of DNA molecules necessary for their aggregation in water–salt solutions. The

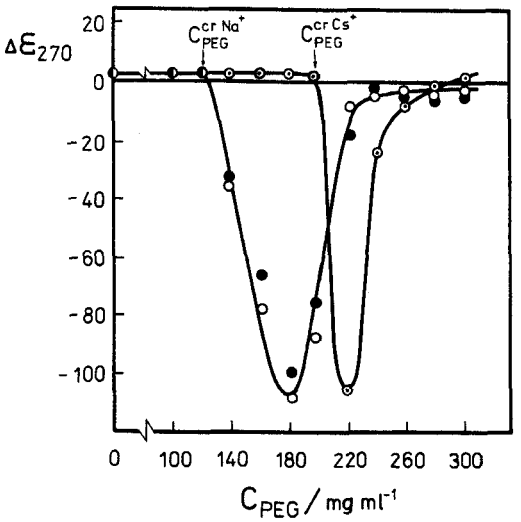

Figure 4. The amplitude of the negative band in the CD spectrum of DNA dispersions ($\Delta\varepsilon_{270}$) formed in solutions of various salts (0·3 M) as a function of C_{PEG} (molecular mass of PEG is 3000). The critical concentrations of PEG (C^{cr}_{PEG}) are shown in the figure. ○, NaCl; ●, $NaClO_4$; ⊙, CsCl.

discrepancy with the theoretical value (88–90 per cent) may simply reflect the existence of differences between the dielectric constants of water–salt and water–salt–PEG solutions.

The band in the CD spectra disappears at a high PEG concentration, although dispersions that scatter light have been formed (see figure 4). So, depending on the nature of the solvent, some dispersions are optically active while other dispersions do not possess such an activity. This observation may be interpreted within the framework of a theory describing the properties of polymeric liquid crystals [18]. In terms of the theory, the twist angle, ϕ, of nematic regions in a cholesteric liquid crystal depends on the ratio of the dielectric parameters of a medium and of the polymer which forms the liquid crystal, as well as on temperature. Proceeding from the interaction energy between polymeric molecules to an expression for the pitch of a cholesteric helix gives

$$\phi = 2\pi d/P = \frac{A\{\varepsilon_m^2 + \varepsilon_m(\varepsilon_\parallel - 3\varepsilon_\perp) + \varepsilon_\parallel \varepsilon_\perp\}, \{(2g_\parallel - g_\perp)\varepsilon_m - g_\perp \varepsilon_\perp\}}{\varepsilon_m(\varepsilon_m + \varepsilon_\perp)^3} - kTB \qquad (3)$$

or

$$\phi = Af\{\varepsilon_\parallel/\varepsilon_m; \varepsilon_\perp/\varepsilon_m\}kTB, \qquad (4)$$

where d is the mean distance between the axes of polymeric molecules in quasi-nematic regions; P is the pitch of the cholesteric helix; ε_m is the dielectric constant of the medium; $\varepsilon_\parallel$, $\varepsilon_\perp$ are the longitudinal and transverse components of dielectric permittivity of a polymeric molecule; $g_\perp$, $g_\parallel$ are the transverse and longitudinal components of the gyration (optical rotation) tensor for the polymeric molecule; B, A are arbitrary constants. Several practical conclusions follow from this equation [12]:

(1) If the solution temperature and the properties of the polymer ($\varepsilon_\parallel$, $\varepsilon_\perp$) are kept constant, ϕ becomes zero when ε_m equals a critical (ε_m^*) value. In other words, if the properties of the solution correspond to ε_m^* then optically active polymeric molecules will not form cholesterics, but a compensated structure [27] whose properties are similar to those of nematics. It has been shown by comparing the properties of PEG solutions, the data from X-ray analysis for DNA liquid crystals and their textures [1], that the value of ε_m^* is about 60.

(2) If the values of $\varepsilon_\parallel$, $\varepsilon_\perp$ and ε_m are fixed, then it is possible, in principle, to reach a temperature at which ϕ also vanishes, giving a compensation nematic. It should be noted that the cholesteric phase can be reformed under particular conditions once a compensated structure has disappeared [27]. Under these conditions, however, the twist of the cholesteric helix formed will have the opposite sign to the original.

(3) If the characteristics of the solvent (T, ε_m) are kept constant and the properties of the polymeric molecules are varied (for example the ratio $\varepsilon_\parallel/\varepsilon_\perp$ is changed), two types of cholesterics differing in the sign of ϕ and one type of nematic can be formed. Therefore, for polymeric lyotropic liquid crystals, it is possible to realize the transition sequence cholesteric$_{(+)}$→nematic→cholesteric$_{(-)}$ caused by changes in the properties of either the solvent or the polymeric molecules.

Provided DNA molecules form cholesteric dispersions and their structure is responsible for the appearance of the band in the CD spectrum, then equation (3) can be applied to describe the properties of these dispersions. The value of ε_m can be changed by varying the PEG concentrations [28]. Indeed, dispersions without an abnormal

optical activity are formed at a high PEG concentration (about 300 mg ml^{-1}) (see figure 4). The value of ε_m can also be changed by adding organic solvents to a solution whose PEG concentration is less than 300 mg ml^{-1}. The formation of dispersions in PEG solutions ($C_{PEG} = 150$ mg ml^{-1}; 0·3 M NaCl) to which methanol, ethanol, isopropanol, morpholine or dioxane have been added [29] allows us to create conditions at which the band amplitude in the CD spectrum passes through zero, and changes sign (see figure 5). Evaluation of ε_m corresponding to the conditions at which the band amplitude in the CD spectrum for a DNA dispersion vanishes (the critical value $\varepsilon_m = \varepsilon_m^*$) shows that ε_m^* ranges from 50 to 57 in different cases [29]. These results are close to the data characterizing ε_m^* determined when the concentration of PEG has been changed in solution. Consequently, dispersions with the nematic rather than cholesteric DNA are formed from optically active DNA molecules under critical conditions. Similar results, which show that dispersions with a different sign of the optical activity can be formed, have been obtained when different hydroxy(ethyleneglycerols) with various molecular masses (>1000) were used as solvents [29]. Hence, the relationship between the dielectric characteristics of the solvent and the DNA is a factor which determines the sense of DNA spatial twisting not only in bulk liquid crystals but also in liquid-crystalline dispersions formed. Obviously, nucleic acids other than DNA will yield dispersions whose optical activity can differ from that of DNA dispersions, even in the same solvent.

The right handed, double stranded synthetic polynucleotides poly(dA-dT)xpoly(dA-dT) and poly(dA)xpoly(dT) (B family), which differ only in the sequence of nitrogen bases in the chains, form dispersions in a PEG containing solution (see figure 6). These dispersions have the same light scattering, but differ in the signs of the intense band in the spectrum. Poly(dG-dC)xpoly(dG-dC) molecules at a high ionic strength

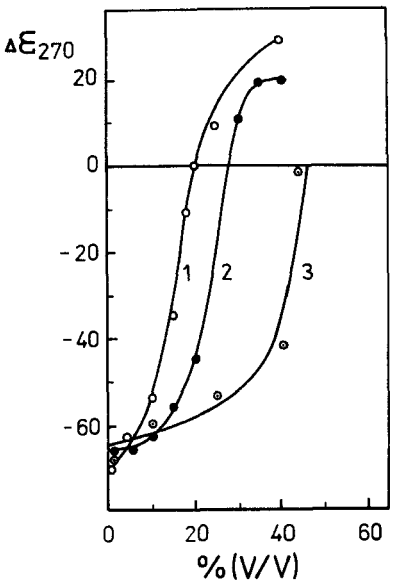

Figure 5. The amplitude of the band in the CD spectrum ($\Delta\varepsilon_{270}$) for dispersions made of DNA molecules in water–salt solutions containing PEG as a function of alcohol concentration (*v*/*v*). (Molecular mass of PEG is 4000; $C_{PEG} = 150$ mg ml^{-1}; 0·3 M NaCl.) Curve 1, isopropanol; Curve 2, ethanol; Curve 3, methanol.

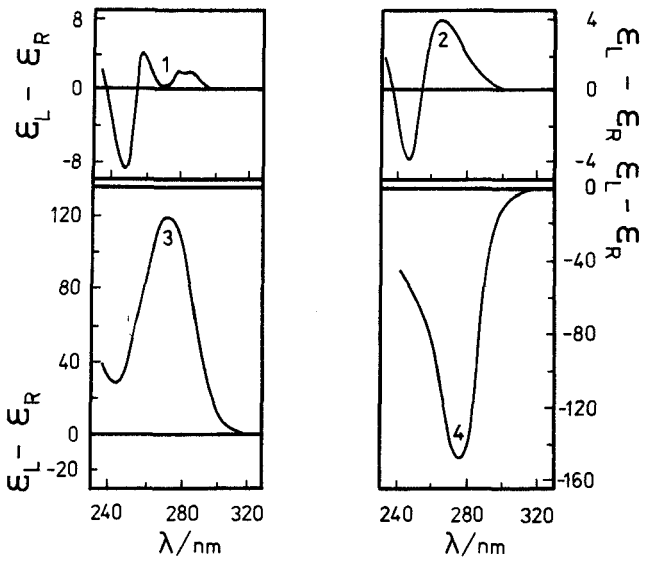

Figure 6. The CD spectra of double stranded, right handed helical synthetic polynucleotides poly(dA)xpoly(dT) (curve 1), poly(dA-dT)xpoly(dA-dT) (curve 2) and their dispersions (curves 3 and 4, respectively) formed in the water–salt–PEG solution. (Molecular mass of PEG is 4000; $C_{PEG} = 180\,mg\,ml^{-1}$, 0·3 M NaCl.)

($\mu > 2{\cdot}6$), belonging to the left handed, helical Z form, also can generate two families of dispersions which differ only in the sign of the intense band in the CD spectrum. The detailed study of dispersions formed from different synthetic, double stranded polynucleotides, performed in [30], showed that for polyribonucleotides (the A family) RNA or poly(I)xpoly(C) molecules create two types of dispersions which differ in the sign of the intense band in the CD spectra. The transition between dispersions which differ in the sign of this intense band can be caused by changing either the PEG concentration in the solution (for RNA) or the ionic strength (for poly(I)xpoly(C)). The double stranded polyribonucleotides poly(A)xpoly(U) and poly(A)xpoly(dT) yield dispersions which have CD spectra with only a positive band under any experimental conditions used [31].

Therefore, nucleic acid molecules belonging to different families (right handed B and A or left handed, Z forms) can be used to create dispersions which are characterized by CD spectra with intense bands in the absorption region of the purine and pyrimidine bases. These data indicate that the existence of an intense band in the UV region of a CD spectrum is not influenced by light scattering. The same factors that govern the structure of nucleic acid liquid crystals in the bulk phase [1] operate at the level of dispersions. We may assume, therefore, that the intense band in a CD spectrum in the region of absorption of the nitrogen bases depends on the character of nucleic acid packing in the dispersions, and that this must be similar to that in cholesterics. Consequently, in spite of their microscopic size, the number of nucleic acid molecules per particle suffices for liquid-crystalline organization.

Another approach to prove the liquid-crystalline nature of the particles in dispersions of nucleic acids is based on the combination of the principles used to derive

equations (1) and (3). Such considerations make it possible to draw the following conclusions:

(1) Equation (1) imposes no limitations on the number of compounds (for example drugs or antibiotics) which can be dissolved in the liquid-crystalline phase of DNA. Hence, at least two bands (rather than one) will appear in the CD spectrum when these compounds interact with DNA and liquid-crystalline dispersions are formed from complexes (DNA–drug or DNA–antibiotic). One of the bands will still be in the region of the DNA nitrogen base absorption while the other will appear in the region (for instance, the visible region) where the chromophores of the coloured compound absorb and light scattering does not effect the shape of the band.

(2) It follows from equation (3), that the interaction of coloured compounds with a DNA molecule can change its dielectric properties both lengthwise and crosswise. Hence, for certain structures of biologically active molecules interacting with DNA, changes in the dielectric properties of the DNA molecules will modify not merely the amplitude of a band in the CD spectrum but also its sign when dispersions are formed from complexes of DNA and biologically active compounds. As an example, figure 7 shows the CD spectra for dispersions made of DNA molecules complexed with daunomycin, a coloured anthracycline antibiotic, in PEG containing solutions. Three series of observations are noteworthy here.

   (i) The CD spectra of the dispersions contain two bands. One occurs in the absorption region of the DNA nitrogen bases ($\lambda \approx 270$ nm) and the other lies in the absorption region of the antibiotic chromophore ($\lambda \approx 500$ nm). Both bands have a negative sign when the extent of binding to the DNA molecules by the antibiotic is low ($r \approx 0{\cdot}04$).
   (ii) The shapes of the bands in the CD spectrum are identical to those in the absorption spectra of the antibiotic and the DNA.
   (iii) Both bands have similar amplitudes (as calculated per mole of DNA base pairs, $\Delta\varepsilon_{270}$, or per mole of the antibiotic bound to DNA, $\Delta\varepsilon_{505}$).

The appearance of two bands in different regions of the CD spectrum can be explained only in terms of dispersions containing cholesteric DNA. The identical sign of the bands indicates that the daunomycin molecules lie parallel to the DNA bases. Dispersions of DNA–daunomycin complexes formed at a high PEG concentration (over 200 mg ml^{-1}) contain no abnormal bands either in the region of DNA or daunomycin absorption.

As daunomycin molecules are complexed (intercalated) between the DNA base pairs, they modify the structure of the DNA and, hence, the energy of interaction between the neighbouring DNA molecules. According to equation (3) this alteration can affect the packing mode of the DNA. Indeed, the negative signs of both bands in the CD spectrum become positive at $r \approx 0{\cdot}1$. The simultaneous alteration of both bands demonstrates unequivocally that the sense of twist of the cholesteric helix is changed by the daunomycin. It was shown in [32] that reversal of the band depends strongly on the peculiarities of chemical structure of anthracyclines, but the correlation between the structure at the reversal conditions was not established.

The identical sign of the CD bands and constant position of their maxima under changes in the surrounding conditions implies that the band of selective reflection

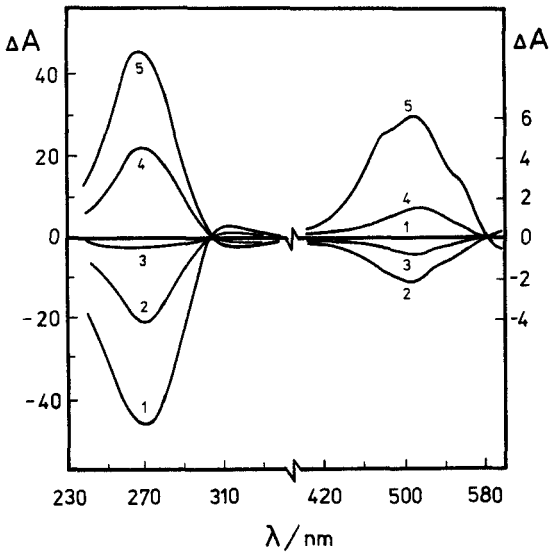

Figure 7. The CD spectra in UV and visible regions for dispersions formed from DNA–daunomycin complexes at different levels of binding of daunomycin to DNA. (Molecular mass of PEG is 4000; $C_{PEG} = 150\,mg\,ml^{-1}$; 0·3 M NaCl.) Curve 1, $r = 0$; Curve 2, $r = 0{\cdot}085$; Curve 3, $r = 0{\cdot}116$; Curve 4, $r = 0{\cdot}135$; Curve 5, $r = 0{\cdot}197$. r is the ratio of concentration of bound antibiotic to DNA bases. (ΔA in cm; $1\,cm = 10^{-4}$ optical units.)

associated with the pitch of the cholesteric helix typical of DNA must occur at wavelengths on one and the same side from both bands in the CD spectrum. Its most probable location is at $\lambda > 500\,nm$, i.e. P must be several microns, which is consistent with the data chracterizing P for DNA liquid crystals ($P \approx 2$–$3\,\mu m$).

It is important to stress that the change of the sign for only one of the two bands may be easily interpreted a quite different way, i.e. in terms of a strong decrease of P characteristics of the DNA cholesteric liquid crystals without alteration of their helical twisting. We can add that the effect of the sign reversal of the band in the CD spectra for dispersions formed from DNA–daunomycin complexes was first demonstrated in 1981 ([32], including the other six anthracyclines differing in chemical structures), carefully studied in 1983 [33] and successfully repeated in 1991 [34].

Provided equations (1) and (3) are applicable to nucleic acid dispersions, we may predict a complicated variation with temperature. Figure 8 illustrates the effect of temperature on the amplitudes of the CD bands recorded when the dispersions of the DNA–daunomycin complexes were heated. The amplitudes of both negative bands decrease when the dispersions formed at low r values ($r \approx 0{\cdot}1$) were heated from 20 to 80°C. At large r values ($r > 0{\cdot}1$), the amplitudes of both positive bands decrease, become zero and then negative, after which they again turn to zero. For DNA saturated with the antibiotic, the amplitude of the positive band drops to zero in one step over the temperature range 75 to 87°C. These results indicate that temperature exerts a greater effect on DNA dispersions than on bulk samples of mesophase. The complicated pattern observed on heating DNA–daunomycin dispersions results from the fact that the structures of liquid crystals and dispersions are governed by factors whose effectiveness in these two cases is different. Apparently, the effect of temperature is associated with the energy contribution caused by the surface tension of dispersion

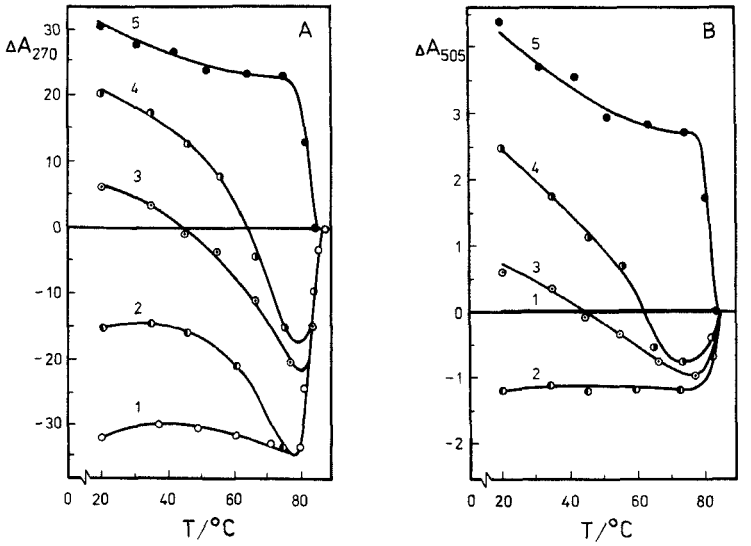

Figure 8. The dependence of the band amplitude in UV (A) and visible (B) regions for the CD spectra of dispersions formed from DNA–daunomycin complexes at different levels of daunomycin binding to DNA. (Molecular mass of PEG is 4000; $C_{PEG} = 170\,mg\,ml^{-1}$; 0·3 M NaCl.) Curve 1, $r = 0$; Curve 2, $r = 0{\cdot}082$; Curve 3, $r = 0{\cdot}126$; Curve 4, $r = 0{\cdot}141$; Curve 5, $r = 0{\cdot}198$.

particles. Therefore, the characteristics of water–polymeric solutions and of DNA molecules are factors which govern the properties of nucleic acid dispersion. Just as in liquid crystals, dispersions of nucleic acids can have a cholesteric or nematic organization. However, for dispersions, the combination of 'governing' factors is more flexible and, this in particular, makes it possible to form dispersions which differ in their properties within a broader range of conditions.

4. Biosensors based on liquid-crystalline dispersions of double stranded nucleic acids

Since the first steps have been taken in the creation of biosensing units on the basis of liquid-crystalline dispersions of nucleic acids, we shall only illustrate the principal aspects of constructing biosensing units.

'Biosensors are an analytical device composed of a biologically sensitive element (an enzyme, an antibody, DNA, cellular organelles, whole cells, or even pieces of tissue) linked to a transformer (electrochemical, optical, calorimetric, or acoustic)' [35]. This definition shows, that in order to construct biosensors we must solve two problems belonging to different fields of science: (1) to construct a biologically sensitive element (a biosensing unit) which recognizes biologically active compounds with the maximal effectiveness (this problem can be solved by biologists); (2) the creation of an adequate scheme in order to record a signal that appears in the system (this problem requires the knowledge of technology).

To construct a biosensing unit it is convenient to make use of dispersions of lyotropic liquid crystals of nucleic acids [36]. The results considered here demonstrate some features of liquid-crystalline dispersions of nucleic acids that are important for the construction of biosensing units. The first is that these molecules tend to form dispersions with cholesteric ordering of the molecules, and hence, with abnormal

optical activity. The second feature is that the spatial ordering of the nucleic acid molecules in liquid-crystalline dispersions does not impair their ability to respond to a specific structural addressing of the biologically active molecules reacting with nucleic acids. Finally, a so-called pseudo-capsulated form of liquid-crystalline dispersions of nucleic acids, i.e. a dispersion immobilized in a polymeric film, has been prepared [37].

Obviously, for biologically active compounds which are capable of forming strong complexes with DNA, i.e. for the compounds that are recognized by DNA, equations (1) and (2) demonstrate than an intense band in the CD spectrum in the absorption region of the dye should appear upon complex formation with cholesteric DNA. If the value of α is 90°, the sign of this band will coincide with the sign of the band typical of liquid-crystalline DNA dispersions. If the dye is located on the DNA molecule so that the value of the angle α is 0°–54°, equations (1) and (2) demonstrate that the intense band in the CD spectrum may have a sign that is opposite in sign to the band characteristic of the liquid-crystalline DNA dispersions. The greater the concentration of the DNA-bound compound, the higher the amplitude of the band in the CD spectrum in the absorption region of the dye (under conditions such that the structure of DNA molecules is not destroyed upon binding the dye). This consideration means that the appearance of intense bands in the CD spectrum of cholesteric dispersions prepared from DNA molecules after adding biologically active compounds allows us to detect not only the presence of a compound in solution and to estimate its concentration, but also to establish the mode of binding of the compound with the DNA molecule. This statement forms a background for creating biosensing units for the detection of coloured biologically active compounds forming strong complexes with DNA.

Using data presented in [36, 38], we shall illustrate the application of a biosensing unit on the basis of DNA liquid-crystalline dispersions for the detection of two antitumor compounds differing in their chemical structures. In figure 9 the CD

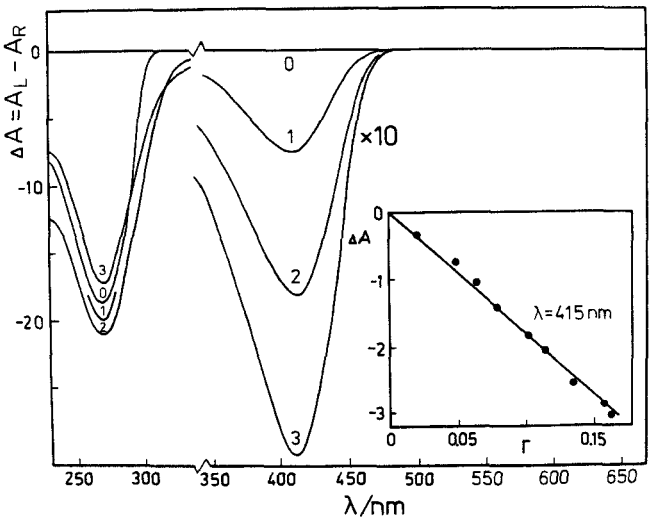

Figure 9. The CD spectra of liquid-crystalline dispersions formed from DNA–BS complexes. Curve 0, $r=0$; Curve 1, $r=0{\cdot}049$; Curve 2, $r=0{\cdot}102$; Curve 3, $r=0{\cdot}163$. (Molecular mass of PEG is 4000; $C_{PEG}=150$ mg ml^{-1}; 0·3 M NaCl.) (ΔA in cm; 1 cm $=10^{-4}$ opt. units.) Inset is the depence of the amplitude on the band in the CD spectra ($\lambda_{max}=415$ nm) of liquid-crystalline dispersions of the DNA–BS complexes versus r.

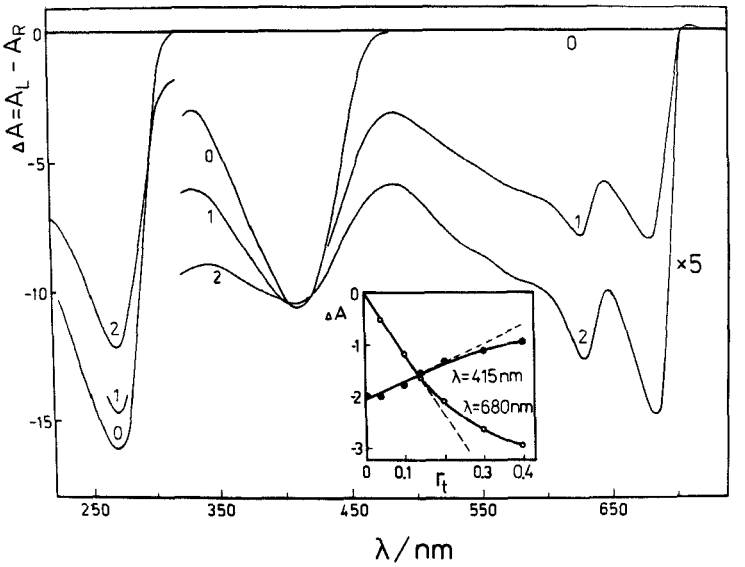

Figure 10. The CD spectra of liquid-crystalline dispersions formed from the DNA–BS complex after addition of MX. Curve 0, r_t BS = 0·202, r_t MX = 0; Curve 1, r_t BS = 0·202, r_t MX = 0·14; Curve 2, r_t BS = 0·202, r_t MX = 0·4. (Molecular mass of PEG is 4000; C_{PEG} = 170 mg ml^{-1}; 0·3 M NaCl.) r_t is the relation of molar concentration of BS and MX to molar concentration of DNA nitrogen bases. Inset is the dependence of the amplitudes of the bands (λ_{max} = 415 nm and λ_{max} = 680 nm) in the CD spectra of liquid-crystalline dispersions of the DNA–BS complexes with added MX versus r_t.

spectrum of the liquid-crystalline dispersion formed from double stranded DNA molecules is compared with the CD spectrum of the same dispersion after adding the antitumor compound 9,10-anthracenediacarboxyaldehyde-bis[(4,5-dihydro-1H-imidazol-2-yl)-hydrazone)dihydrochloride (bisantrene hydrochloride, BS) [39]. After adding BS, the amplitude of the DNA negative band ($\lambda_{max} \approx 260$ nm) in the CD spectra does not change significantly (curves 1–3). Addition of BS is accompanied by the appearance of a negative band located in the absorption region of BS ($\lambda_{max} \approx 415$ nm, curves 1–3). The appearance of an intense band in the CD spectrum in the absorption region of BS demonstrates that this compound is present in solution. The negative sign of the band in the CD spectrum in the absorption region of BS proves that BS molecules form a complex with the nitrogen bases, and that they are located on the DNA molecules such that the angle of inclination α is about 90°; this is possible when the BS molecules are intercalated between the nitrogen bases. Finally, the experimentally measured amplitude (ΔA) of the negative band in the CD spectrum in the absorption region of BS is directly proportional to the concentration of BS molecules bound to DNA (inset in figure 3). Since there is a definite correlation between the concentration of BS molecules in solution (r_t) and the concentration of DNA-bound BS molecules (r), the results obtained (see figure 9) imply that the biosensing unit based on liquid-crystalline DNA dispersions allows us to establish the presence and concentration of BS in solution, and also the mode of binding of BS molecules to the DNA molecule.

Figure 10 shows the CD spectra of liquid-crystalline dispersions prepared from (DNA–BS) complexes after adding another synthetic antitumor derivative of the

anthracycline group-1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedion (mitoxantrone, MX) [40]. We can see that negative bands are present in the absorption region of DNA ($\lambda_{max} \approx 260$ nm, curves 0–2), BS ($\lambda_{max} \approx 415$ nm, curves 0–2), and also MX ($\lambda_{max} \approx 680$ nm, curves 1–2). Despite a very complex situation i.e. the presence of two different drugs, the latter band demonstrates that MX molecules intercalate between the bases. The dependence of the amplitudes of the two last bands on the total concentrations (r_t) of added drugs (inset in figure 10) demonstrates that computer calibration which accounts for the displacement of one dye from the DNA complex when another dye is added to the biosensing unit is necessary in this case. Figure 10 shows that the biosensing unit recognizes both BS and MX molecules in a complex mixture.

Thus, biosensory based on liquid-crystalline DNA dispersions can be used for analytical purposes. The minimum concentration of dyes which they can detect at present is about 10^{-7} M.

The application of liquid crystals of nucleic acids is an effective method that allows us to determine biologically active compounds which differ in their chemical structure and in the mechanisms of interaction with nucleic acids. We expect that once the technical problem is solved, namely, convenient portable signal transformers (optical, first of all) are created and microelectronic devices are constructed, such biosensors will make it possible to detect specific types of biologically active compounds. These biosensors will be suitable for both laboratory and personal use.

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