Novel Cholesteric Phase
in Dispersions of Nucleic Acids
due to
Polymeric Chelate Bridges

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Abstract

We consider cholesteric liquid-crystalline DNA dispersions, and show that polymeric (Dau-Cu) complexes, the so-called bridges, between pairs of DNA molecules may generate a super liquid-crystalline structure (BR-phase). The latter could have a layered spatial structure and an abnormal optical activity that could have a bearing upon the intense CD-band observed in DNA-dispersions.
1. Introduction

The existence of the liquid-crystalline (LC)-phases of DNA molecules complexed with antibiotics of the anthracycline family opened a new avenue to studying both DNA and solvents. In this respect circular dichroism (CD)-experiments using daunomycin (Dau) are very interesting, [1], [2], [3], owing to the (Dau-DNA) complexes having very specific structural properties and optical activity. It is alleged, [4], [5], that Dau-molecules may intercalate between base pairs of the double-stranded DNA fixed in liquid-crystalline dispersion, forming the so-called intercalation complex (I), and generate an additional set of chromophores contained in a DNA-molecule, that is besides the original ones due to nitrogen bases absorbing in the UV region of 270 nm, there emerge chromophores, belonging to Dau-molecules, that absorb in the region of 500 nm, see [4], [6], [7] for the detailed analysis of optical phenomenon involved. As the concentration of daunomycin increases, the intensity of the CD-band at 500 nm rises, until it reaches a saturation point. According to [5] the saturation of the CD-intensity corresponds to the ratio of one Dau molecule per five base pairs, see Fig.1. Further adding the daunomycin results only in a larger number of Dau molecules that are not attached to DNA molecules. These free Dau molecules do not produce a coherent effect as regards the CD absorption, and therefore do not have a bearing upon the CD intensity, in agreement with the saturation effect mentioned above. It is worth noting that one can change the sign of both CD bands, [1], at 270 nm and 500 nm by increasing the concentration of Dau above a certain point. Since the sign of CD band corresponds to the sense of the helical twist, i.e. the left/right one for the negative/positive band, respectively, one may conclude that Dau molecules generate a structural transition of the cholesteric DNA phase.

The conformation of (DNA-Dau) complex is substantially modified, if the dispersion is treated with CuCl$_2$, and therefore copper ions, Cu$^{2+}$, are added in the system. Then according to the X-ray analysis, [5], the Dau-saturation discussed above having been reached, there appear Dau-molecules, adsorbed at the surfaces of the DNA-double helices fixed in the cholesteric phase, the so-called external complex II, see Fig. 2. If the concentration of Cu$^{2+}$ is large enough, they result in the formation of chelate complexes. In case the chelate complexes are properly located with respect to the double helices of adjacent DNA molecules, there arise certain planar structures, or bridges, that cross-link pairs of adjacent DNA-molecules, [5], see Figs. 1—3, [6]—[11]. It is generally accepted that the bridges have the form of polymeric chelate complexes

\[ [DNA - Dau - (Cu - Dau - Cu)_n - Dau - DNA] \] (1)
The amplitude of CD-bands increases tens times after the Dau - and the copper concentrations having reached threshold values, see Fig.4. Here it should be noted that for one thing isolated Dau-molecules, as well as other isotropic aggregates, are not sufficient to produce an abnormal optical activity, and for another there are relatively few Dau-molecules that are properly oriented so as to form chelate bridges. Thus, even though according to the arguments given above, the thresholds could correspond to the formation of the chelate bridges, the total effect due to Dau-molecules and their chelate complexes considered separately could be too small. Therefore, it is reasonable to suggest that the observed increase in the CD-bands be due to a cooperative effect related to chelate bridges, and one should look for it in their spatial organization. Even though the anisotropy of the chelate bridges is small, they could acquire an orientational ordering while serving as a kind of linker between DNA-molecules. Thus, we may suggest that cholesteric dispersions of DNA doped with Cu$^{2+}$ could be visualized as two LC systems in interaction; the DNA molecules forming the dominant part of them.

2. Double Cholesterical Phase

In what follows we shall not take into account the influence of ambient solvent on DNA molecules and polymeric chelate bridges. We may cast the arguments given in the Introduction in a more quantitative form by employing the familiar macroscopic formalism of the order parameter and assign a unit vector $\vec{n}$ to the cholesteric phase formed by the DNA-molecules, and a unit vector $\vec{\nu}$ to the bridges. Initially, we do not make any assumption about their forming a nematic or cholesteric phase; their orientational ordering is to a large extent determined by the molecules of DNA. Therefore, we assume that the free energy of the compound system formed by DNA and bridges, should contain an interaction term given by the following equation

$$F_I = \int d^3r \lambda (\vec{\nu} \cdot \text{rot} \, \vec{n})^2$$

in which the real unit vectors $\vec{n}, \vec{\nu}$ are the order parameters for the DNA and the bridges, respectively. It is important that the expression given above is the simplest one for the interaction energy that takes into account the cholesteric twist of the phase due to DNA-molecules, and its possible interaction with bridges. Next, we may write down the part of the free energy related to the
DNA-molecules considered as a separate system, and since we aim at deriving only a qualitative model, we may employ one dimensional approximation and write

\[ F_{DNA} = \int \frac{k}{2} (\vec{n} \cdot \text{rot} \vec{n} - q_0)^2 \, dz, \] (3)

that is the usual form accommodating cholesteric twist.

Again employing the most simple terms, we assume that the bridges have only the orientational energy, proper, the cholesteric effects being due to their interaction with the DNA-molecules; therefore, we write

\[ F_{Br} = \int \frac{\mu}{2} \left( \frac{d}{dz} \vec{\nu} \right)^2 \, dz \] (4)

On combining equations (3), (3), and (4), we obtain the equation for the free energy of the whole system

\[ F = \int \left[ \frac{k}{2} (\vec{n} \cdot \text{rot} \vec{n} - q_0)^2 + \frac{\mu}{2} \left( \frac{d}{dz} \vec{\nu} \right)^2 + \lambda (\vec{\nu} \cdot \text{rot} \vec{n})^2 \right] \, dz \] (5)

in which the z-axis is assumed to be the helical one of the DNA-cholesteric, so that the vector \( \vec{n} \) can be cast in the form

\[ \vec{n} = (\cos \phi, \sin \phi, 0) \] (6)

in which the polar angle \( \phi \) is a function of \( z \) and

\[ \frac{d\phi}{dz} \]

is the twist angle. The vector \( \vec{\nu} \) reads

\[ \vec{\nu} = (\sin \theta \cos \psi, \sin \theta \sin \psi, \cos \theta) \] (7)

The angle \( \theta \) is, to a certain extent, a joint characteristic of the position of adjacent DNA-molecules and chelate bridges linking them. In fact, let us consider two molecules of DNA which we may visualize as two skew straight lines, \( \Lambda_1 \) and \( \Lambda_2 \), at a distance \( d \) from each other; \( d \) being equal to the distance between two parallel planes, \( \Pi_{1,2} \), that contain the lines \( \Lambda_{1,2} \), respectively, see Fig.5. If the length of a bridge, \( l \), and the distance \( d \) are fixed, the angle \( \theta \) is fixed as well. In real life, there are fluctuations of the positions of the planes \( \Pi_{1,2} \) that produce a change in \( d \). Also, the length of bridges may vary, for
the structural form given by Eq.(1) may correspond to different values of the number of Cu-units, \(n\). According to X-ray analysis \[2\], in the absence of the bridges, the change in \(d\) amounts to 1%. According to a crude and qualitative nature of our model we shall consider only sufficiently small fluctuations of \(d\), and assume the angle \(\theta\) fixed.

Thus, let us assume that \(\theta = \theta_0 = \text{const}\). Then the equation for the free energy (5) acquires the form

\[
F = \int \left[ \frac{k}{2} (\dot{\phi} - q_0)^2 + \frac{\mu}{2} \theta_0 \dot{\psi}^2 + \lambda \sin^2 \theta_0 \cos^2 (\phi - \psi) \dot{\phi}^2 \right] \, dz \tag{8}
\]

in which the dot means the differentiation with respect to \(z\). The minimization of equations for the free energy given by Eq.(8) reads

\[
\ddot{\phi} = \frac{\lambda \sin^2 \theta_0 \sin[2(\phi - \psi)]}{k + 2\lambda \sin^2 \theta_0 \cos^2 (\phi - \psi)} \left(2\dot{\phi}\dot{\psi} - \dot{\phi}^2\right) \tag{9}
\]

\[
\ddot{\psi} = \frac{\lambda}{\mu} \sin[2(\phi - \psi)] \dot{\phi}^2
\]

If there are no bridges, i.e. \(\mu = \lambda = 0\), we have the usual solution \(\dot{\phi} = q_0\) corresponding to the minimum of \(F\) and giving the cholesteric structure. We assume that the terms related to the bridges are small in comparison with the main cholesteric term

\[
\frac{k}{2} (\dot{\phi} - q_0)^2,
\]

and measure the constants \(\nu\) and \(\lambda\) in units of \(k\); the latter being of order \(10^{-6}\) dyn. There is no experimental information about the values of \(\mu\) and \(\lambda\); our guess is that for one thing \(\mu\) is much smaller than \(k\), \(\mu \ll k\), owing to a small anisotropy of the molecules of bridges, at least in comparison with those of DNA, and for another the magnitude of \(\lambda\) approaches that of \(k\), even though being smaller, for the interaction term should be strong enough to drive the bridges along the orientational twist of DNA molecules. In fact, one can visualize a chelate bridge as a planar rectangle with the ratio of the sides about 3 by 10 Å, so that its anisotropy is much smaller than that of DNA-molecules. Therefore, we shall take \(k = 1\) and \(\mu\) of the order of 0.01 and \(\lambda\) of 0.1, in the units given above.

As regards functional (8), we do not have the ambition to look for its minimum, but even studying the necessary condition for the latter, provides valuable information about the structure of the system. In fact, there is an exact solution to the minimization equations for (8), but its formulas are not particularly tractable, and it is more practical to employ computer, from the very beginning. For our purpose we need to consider the behaviour of the twist angles, that is \(\dot{\phi}\) for the DNA-molecules, and \(\dot{\psi}\) for the bridges. To understand their
possible configuration, let us visualize two adjacent molecules of DNA as two skew straight lines, see Fig.5. It is easy to convince oneself that the locus of points for the middle of the segment AB of a given length \( l \) is a rather elongated ellipse \( E \), see Figs. 6 and 7.

It is worth noting that the ellipse is "flat" proportionally to the smallness of the twist angle \( \Phi \) between the skew lines \( \Lambda_{1,2} \). The z-axis being the rotation axis of the helical structure for the DNA-cholesteric, the straight lines \( \Lambda_{1,2} \) rotate about \( O_2 \) at the angular velocity \( \dot{\varphi} \), while an observer moves along \( O_2 \). In the meantime the segment AB, corresponding to a bridge, participates in two motions; that of the straight lines, and a motion of its own along the ellipse \( E \). The latter has its own structure as to the sign of the motion along \( E \) and characteristic time, or period. Of course, we do not mean the motion in time, our problem is the statical one, and there is no time, but only a change in the characteristic of the system as we move along the helical axis, \( O_z \), which is the rotation axis of the cholesterical structure. It is important that the spatial conformation of the bridges and the DNA-molecules is determined by equilibrium conditions imposed on the system, and is of thermodynamical nature.

Thus, our hypothesis is that the orientational ordering of the bridges could substantially differ from that of the DNA-molecules. The numerical simulation of Eqs. supports the claim.

The behaviour of the twist angles \( \dot{\varphi} \) and \( \dot{\psi} \) is illustrated in Fig. 8. We see that they can differ in magnitude several times, and in sign as well. We can visualize the phenomenon with the help of Figs.6 and 7 for the motion of the segment AB, or bridge, round the supporting ellipse \( E \). In fact, the comparison of the numerical simulation given in Figs. 8, and Figs.4,5 indicates that the periods during which the twists \( \dot{\phi} \) and \( \dot{\psi} \) are almost identical correspond to the location of the segment AB, or bridge, at the aphelion and the perihelion of the ellipse \( E \). On the contrary, the flat regions of the ellipse correspond to the motion of the bridge at its own angular velocity, which is several times larger than that of the twist \( \dot{\phi} \). It should be noted that we do not mean an actual motion in time, but only the change in variables as we move along the Oz-axis.

Thus, we may expect that in a dispersion of DNA cross-linked by chelate bridges there is a super helical structure due to the bridges, which is in a way superimposed on the cholesteric one of DNA molecules, see Fig. 9. In fact, the numerical simulation shows that there is a layered orientational structure of bridges that has the property of a cholesteric with a variable twist angle. It admits of the abnormal CD-band discussed in the Introduction. In fact, it is alleged that the absorption strongly depends on the twist angle of the cholesteric structure, and therefore, the cholesteric twist for bridges being several times larger than that for DNA, one may expect that the CD-band could be larger than for the cholesteric phase of DNA in the absence of them.
3. Conclusion: cholesterical BR-phase

We suggest that in dispersions of DNA containing chelate bridges there could exist, besides the cholesterical phase of DNA molecules, another cholesterical phase generated by the bridges, which we shall call the BR-phase, see Fig.9. The latter could be the cause for the abnormal optical activity observed in CD-spectra in the region of 500 nm, and should have a layered structure.

It should be noted that, as follows from our crude model, the cholesteric pitch of the DNA dispersion does not change under the influence of the bridges, at least for the values of parameters considered in this paper. The circumstance has an important bearing upon the optical properties of DNA dispersions. In fact, the intensity of the CD band at 270 nm, which corresponds to DNA chromophores, should have remained unaltered in the presence of the bridges, but it increased. Therefore, we shall conclude that the intensity of the CD bands depends also on certain factors besides the orientational ordering of the bridges. Perhaps, the chelate bridges could change the coupling of oscillating dipole moments of adjacent DNA molecules so that collective exciation modes should emerge and result in the observed large intensity of the CD bands.

In this respect it is worth noting that the use of ions of different transition metals, e.g. cadmium or nickel, instead of copper, results in different properties of the BR-phase, as regards, at least, its optical activity. The phenomenon could have an important bearing upon the study of DNA-dispersions and serve as a kind of probe into the physics of DNA and solvents. For example, it would be extremely interesting to find compounds capable of changing the sign of CD-absorption band; the picture given above of a chelate bridge "moving" along the supporting ellipse in the opposite direction with respect to the cholesteric twist of DNA, suggests that it could be possible.
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