The Chirality of the Cholesteric Phases of DNA and G-Wires: Its Connection to their Molecular Structures

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Abstract: While the handedness of the cholesteric phases formed by assembled guanosine derivatives (G-wires) follow the correlation right-handed helices → right-handed cholesterics (left-handed → left-handed), the cholesteric phase formed by B-DNA (right-handed helix) is left-handed. This apparent discrepancy is overcome by considering pitch (p) variations with temperature. Plots of p^{-1} versus T^{-1} have, in fact, the same trend (positive intercept and negative slope) in the case of right-handed G-wires and B-DNA, while for the left-handed G-wire of deoxyguanosine monophosphate (dGMP), the opposite behavior is observed (negative intercept and positive slope). Therefore, the relation between molecular handedness and cholesteric helicity cannot, in general, be assessed by using measurements based on a single temperature; hence the temperature variation of the cholesteric parameters should be investigated. In all cases there is no remarkable salt effect on the cholesteric parameters.

Keywords: chirality · cholesteric phases · DNA · G-wires · liquid crystals

Introduction

Both B-DNA and G-mono and oligonucleotides, dissolved in water, can form liquid crystalline phases of the cholesteric and hexagonal type.[1, 2] While for DNA the building blocks of the phases are double helices (in the present discussion only data on B-type DNA, i.e., right-handed double helices, are taken into account), in the case of guanosine derivatives the building blocks of these phases are self-assembled columnar structures based on G-quartets, commonly referred to as G-wires, similar to the four-stranded helix formed by poly(G).[1]

Oligodeoxyguanylates d(GpGpG) (dG_3), d(GpGpGpG) (dG_4), and d(GpGpGpGpGpG) (dG_6) aggregate in isotropic solutions to give right-handed four-stranded helices;[3] on the other hand, deoxyguanosine monophosphates (dGMPs) form left-handed four-stranded helices.[3, 4] These structural assignments were based on circular dichroism (CD) spectroscopic measurements which were used for an empirical correlation with the spectrum of the assembled form of poly(G), known to be right-handed from fiber X-ray work.[5] Subsequently, the assignments were rationalized theoretically with an exciton calculation based on the framework of poly(G).[6] Right-handed, four-stranded helices are characterized by a positive CD signal at about 260 nm and a negative CD signal at about 240 nm.

We were puzzled when we observed that while B-DNA gives a left-handed cholesteric phase at room temperature,[7, 9] the cholesteric phases formed by dG_3, dG_4, and dG_6, in which the columnar aggregates are right-handed, are instead right-handed.[3] The cholesteric phases formed by dGMPs (left-handed, four-stranded helices as seen from the negative CD couplet centred at about 250 nm) are left-handed, as expected.[3, 4]

The configurational assignments of the cholesteric phases were again based on a careful use of CD spectroscopy; the samples used were those with a planar alignment in order to minimize artifacts due to linear anisotropy.[10] The data obtained from the CD spectra of the guanine and the DNA chromophores were confirmed by using an external dye chromophore dissolved in the phases themselves.[3, 8]

The inconsistent fact was that helical structures with the same right-handed chirality (DNA and G-wires formed by dG oligomers) and similar chemical constitution pack to give cholesteric phases with opposite handedness.

Results and Discussion

The structure of the tetramer formed by d(TG_4T) obtained from high-resolution single-crystal X-ray diffraction has been

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reported recently: it is unambiguously a right-handed four-stranded helix.[11] This finding gives us a model which is more reliable than that obtained by the fiber work on poly(G). To confirm our handedness assignments, we have measured the CD spectrum of the assembled species formed by this molecule (Figure 1).

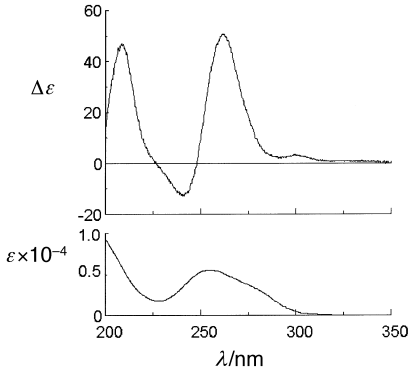

Figure 1. CD spectrum of d(TG_4T) in water ($c = 1.15 \times 10^{-3}$ M, $T = 25\,^{\circ}C$).

The spectrum is nicely characterized by a positive band at about 260 nm and a negative one at about 240 nm, confirming unambiguously the assignments reported previously.

A simple steric model,[10, 12] extended from the Straley model,[13] for the packing of helical structures, which schematizes the situation in the cholesteric phase, is reported in Figure 2. The model is composed of right-handed screws in which the thread is tightly wound (Figure 2a), while the structure represented in (Figure 2b) is considerably unwound with respect to that in a). In a), the *right-handed screws pack in a right-handed way,* while in b), the right-handed screws pack in a left-handed way.[10] More precisely, the packing handedness is connected to the helix angle ϕ of the screws which is $> 45^{\circ}$ in Figure 2a and $< 45^{\circ}$ in Figure 2b; when the helix angle is 45° (the magic angle), the two screws pack at 90° into an achiral structure. For an ideal system, there is a simple relationship between the pitch to diameter ratio and the helix angle. When this ratio is π the helix angle is 45°, for smaller ratios one has the case shown in Figure 2a, while for larger ratios the case shown in Figure 2b.

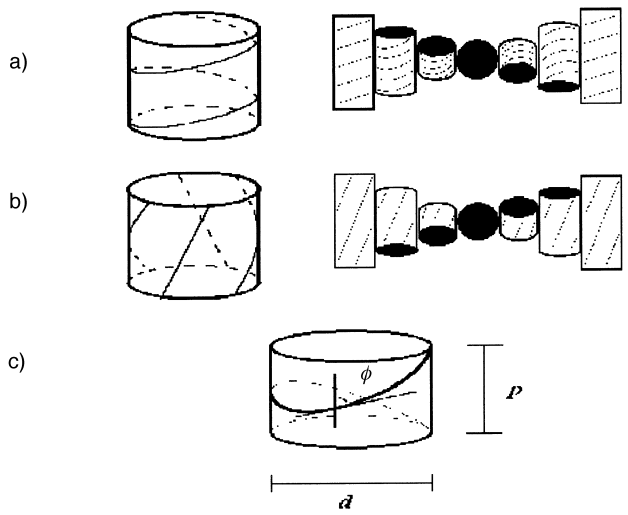

Figure 2. The different handedness of the packing of right-handed helices with different helix angles ϕ. While in a) the pitch-to-diameter ratio is less than π ($\phi > 45^{\circ}$) the superhelix is right-handed, in b) helices with $p/d > \pi$ ($\phi < 45^{\circ}$) generate a left-handed superhelix. c)Schematic diagram that shows the definition of the pitch (p), diameter (d) and helix angle (ϕ)

In his pioneering report,[13] Straley was mainly concerned in showing how chirality can be transferred from molecules to the cholesteric phase by steric effects. The relationships between the handedness of the helical objects and that of the phase was a minor concern. The model in Figure 3 in ref. [13] resembles that of Figure 2b of our report, but with a further simplification. In a more realistic model,[10, 12] the ridges of one screw fit into the groove of the other screw, while in the model given in ref. [13], the axis of the second screw is parallel to the groove of the first screw. In this way right-handed screws always pack with left-handed chirality, independently of the helix angle of the screws. This approximation might apply to screws with a very high pitch-to-diameter ratio, but seems unrealistic for many helical polymers.

We thought that the different packing of DNA and G-wires in the cholesteric phase could be connected to the different geometry of the two structures, associated with values of the helix angle slightly below and above the critical angle of 45° in accordance with the simple steric model reported above. However, a comparison of recent single-crystal X-ray data for four-stranded d(TG_4T)[11] with the classical data of the B-DNA duplex of d(CGCGAATTCGCG) seems to exclude structural differences that could drastically alter the helix angle in the two cases (see Figure 3). In both cases the helix angles are rather similar and $> 45^{\circ}$.

It is known that temperature variations might affect the pitch of the cholesteric phases: the use of cholesteric phases formed by cholesterol derivatives in thermography being emblematic. In some cases, inversion of the helical handedness was also observed;[16] consequently a correlation of helical sense in thermotropic cholesteric liquid crystals (LCs) should be done over an extended temperature range whenever possible.

In particular, in the field of lyotropic cholesteric phases, formed by helical polymers, which is relevant to the present

Abstract in Italian: *Mentre il senso delle fasi colesteriche formate dall'assemblaggio di derivati della guanosina (G-wires) segue la relazione eliche destre → colesterici destri (eliche sinistre → colesterici sinistri), la fase colesterica ottenuta dal DNA (elica destra) è, invece, sinistra. L'apparente contraddizione è superata qualora si consideri la variazione del passo con la temperatura. Le curve di p^{-1} contro T^{-1} mostrano, infatti, lo stesso andamento (intercetta positiva e pendenza negativa) nel caso del B-DNA e dei G-wire destri, mentre nel caso del G-wire sinistro di dGMP, si osserva un comportamento quasi speculare (intercetta negativa e pendenza positiva). Pertanto, la relazione tra la chiralità molecolare e il senso del colesterico non può, in generale, essere ottenuta mediante misure effettuate ad una sola temperature, ma si dovrà investigare la dipendenza dalla temperatura dei parametri del colesterico. In nessun caso si è osservato un effetto significativo dei sali sui parametri colesterici.*

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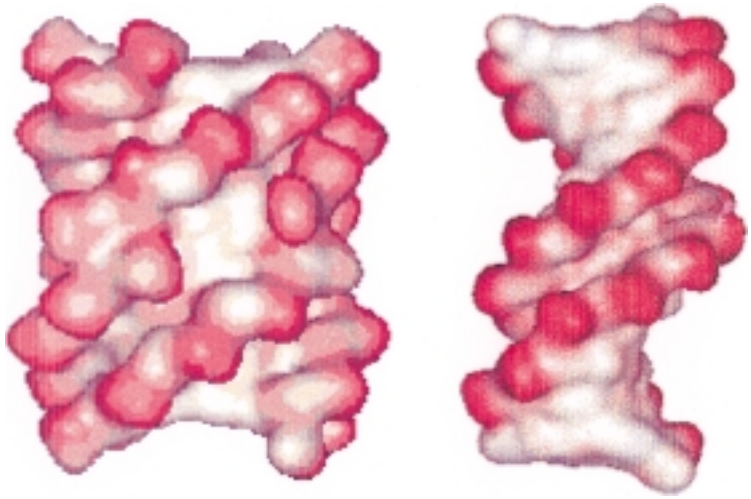

Figure 3. "Soft-Surface" pictures (WebLab ViewerLite 3.10, 1998 by Molecular Simulations) obtained from high resolution X-ray diffraction data of a G-wire formed by d(TG_4T)[14] (left) and a B-DNA duplex of d(CGCGAATTCGCG)[15] (right).

case, Sato, Green et al.[17] have emphasized the importance of the study of pitch (p) variations with temperature and have proposed a simplified treatment to rationalize the phenomenon. The treatment is within the framework of the continuum theory of the cholesteric liquid crystals and uses the Straley approach.[13] It is possible, in contrast to thermotropic systems, because in lyotropic LC the phase boundary is temperature invariant, as predicted by the Onsager theory (within limits of temperature invariance of the cholesteric building blocks). The variation of reciprocal pitch versus reciprocal temperature is correlated to the entropic and enthalpic factors, S_q and H_q, respectively. This treatment allows the experimental estimate of the entropic and enthalpic contributions to the cholesteric twist.

From Equation (1) a plot of the twist (q_c) versus reciprocal temperature should be linear. The intercept S_q is related to the entropic gain by twist, hence to the steric contribution depicted in Figure 2. The slope H_q is related to dispersive forces. This theory has been successfully applied to uncharged helical polymers.[10b, 17–19]

$$2\pi/p = q_c = H_q/T + S_q \qquad (1)$$

We have measured pitch variation with temperature[20] and plotted the data according to Equation (1), even if in the present case we are dealing with polyelectrolites for which strong intermolecular non-specific and specific interactions have been observed.[22, 23]

The plots for the cholesteric phases of B-DNA and of the G-wires formed by dG_4 and deoxyguanosine 5′-monophosphate (5′-dGMP) are reported in Figure 4. The plots are linear in a consistent temperature interval and, although the absolute values of the slopes are rather different (see caption to Figure 4), the trends for B-DNA and the G-wire of dG_4 (right-handed building blocks) are similar and that of the G-wire formed by 5′-dGMP (left-handed building blocks) is opposite to the first two, which reflects the opposite handedness of the cholesteric phase. The negative handedness of the cholesteric phase of B-DNA at room temperature does not reflect the chirality of the polymer, while the temperature dependence of the twist does. Temperature dependences with positive intercept and negative slope are correlated to right-handed polymers, while those with negative intercept and positive slopes are correlated to left-handed ones. In general, handedness correlations in lyotropic cholesteric phases based on data at a single temperature are not reliable, and the investigation must be carried out on a large temperature range.

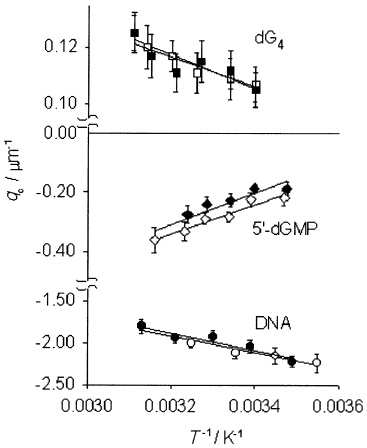

Figure 4. Plots of q_c versus T^{-1} for cholesteric aqueous solutions of short fragments of DNA, 5′-dGMP, and dG_4; filled symbols refer to heating scans, while open symbols to cooling scans. H_q and S_q values obtained from Equation (1) are -1027 ± 160 and $+1.39 \pm 0.55$ for DNA, $+516 \pm 80$ and -1.98 ± 0.27 for 5′-dGMP, and -56 ± 12 and $+0.31 \pm 0.04$ for dG_4, respectively.

It was reported that the cholesteric pitch formed by short DNA (ca. 130 base pairs (bp)), as for the present study, is independent from the ionic strength of the solution.[24] This observation, which remains unexplained, seems to indicate that the effect of charges on the cholestric twist is small. The twist can be considered without adopting a larger diameter of the polymer to compensate for the charges of the phosphate groups.

We have measured the effect of the ionic strength on the G-wire cholesteric phases of dG_4 and 5′-dGMP in the full temperature range (Figure 5).

There is no evident effect and the plots are identical within experimental error.

We could therefore attempt to use the parameters of Equation (1) to interpret our data. The plots for DNA and dG_4 have positive intercepts (S_q) and negative slopes (H_q), while that for 5′-dGMP has a negative intercept and a positive slope (see caption of Figure 5).[25] Therefore, both steric S_q and enthalpic H_q contributions are important in determining the cholesteric helical parameters. The sign of the steric contribution is related to the model of Figure 2.

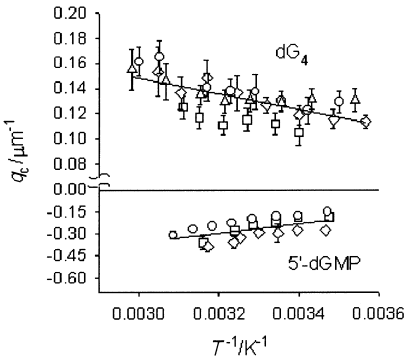

Figure 5. Plots of q_c versus T^{-1} for cholesteric aqueous solutions of 5′-dGMP and dG_4 in the presence of different amounts of NaCl: square, diamond, circle and triangle indicate NaCl concentrations of 0, 0.1, 0.2, and 0.5 M, respectively

The experimental steric entropic contributions S_q, which is positive for the packing of right-handed helices and negative for left-handed helices, seem to indicate that in our examples the steric model of Figure 2a is followed, that is right-handed screws pack in a right-handed superstructure. This finding is perfectly compatible with the geometrical features of both DNA and G-wires (Figure 3).[26]

In conclusion, the discrepancy between the handedness of the cholesteric phases of B-DNA and of G-wires vanishes when the temperature dependence of the pitches is considered. The important point is that whenever one is comparing the handedness of lyotropic cholesterics, the pitch versus temperature dependence should be studied. The insensitivity of pitch values to changes of the ionic strength of the solutions seems to indicate that Equation (1) is followed, at least qualitatively, also by polyelectrolites.

Experimental Section

Calf thymus DNA (Sigma) fragments were obtained following the procedure described in ref. [8]. The tetramer dG_4 (sodium salt) was synthesized and purified following a previously described procedure.[3] The oligo d(TG_4T) (sodium salt) was obtained desalted from Geneset (France) and used without further purification. The deoxynucleotide 5′-dGMP (ammonium salt) is a commercial product from Sigma.

The DNA samples (32 wt % in water) were prepared by sealing a drop of solution between two cover slides with the commercially available SureSeal system (thickness 0.2 mm) as a spacer. The fingerprint textures of the DNA samples were obtained spontaneously.

Samples of the dG_4 and 5′-dGMP cholesteric phases (15 and 23 wt % in water, respectively) were prepared, sealed in flat glass microslides (Vitrocom, thickness 0.3 mm). The microslides were kept in a magnetic field (0.8 T) for 1 – 2 hours in order to obtain the fingerprint texture.[3] Each sample was observed with a Zeiss polarizing microscope (Standard 16) equipped with a videocamera: a line profile analysis of the picture allows the determination of the pitch of the cholesteric phases (see Figure 6). Variable temperature measurements were carried out with a metal platform thermostatted with water circulation (Linkham), connected to a Neslab circulator thermostat.

CD spectra were recorded with a Jasco J-710 dichrograph. The cholesteric handedness was obtained from the CD spectra.[10] The samples were inserted into sandwich quartz cells with a 0.01 – 0.001 cm path length: observation through a polarizing microscope revealed almost no anisotropy (i.e., the sample between crossed polars was dark for almost the entire cell) and rotation of the sample around the direction of the light beam did not reveal artefacts originating from linear dichroism.

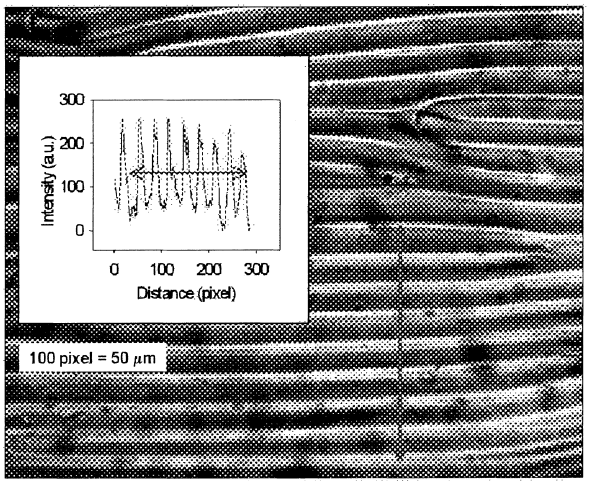

Figure 6. A typical picture of the fingerprint texture (the spacing corresponds to the half-pitch) and the line profile plot leading to the determination of the pitch. The double-headed arrow represents the area of the image under analysis

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[19] Unfortunately a simple model such as that of Figure 2 is not available for the evaluation of the dispersive contribution to the twist.

[20] The melting temperatures (T_m) of the quadruplexes increase strongly with the concentration of the guanine derivatives. Furthermore, added salts increase further the stability of the aggregates. In the case of dG_4, in diluted solution (0.4%) and without added salts, T_m is about 50 °C.[21] Even if it is not possible to measure T_m in the cholesteric phase, the persistence of the phase above 70 °C indicates that the quadruplex is stable up to at least this temperature. In the case of 5′-dGMP in dilute solution (0.4–4%), T_m is much lower; however, the cholesteric phase persists in the interval investigated. The slight curvature observed towards high temperature (data not reported in the figure) could be related to the incipient melting of the quadruplex. In all cases, the plots with and without added salts are very similar; this indicates again that no dissociation of the quadruplexes seems to occur in the temperature range investigated.

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[26] The simplified steric model, derived from Straley and proposed by Sato, implies that a right-handed screw packs with a rod to always give a left-handed superstructure, independent of the helix angle of the screw. This model might be applied to polymers with a very large pitch-to-diameter ratio. In some examples, experimental uncertainties might bring the validity of this model into question; especially in the case of synthetic polymers.[10]

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