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Oligodeoxyguanylates: A case of self-assembly leading to lyotropic liquid crystals

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Abstract. The guanosine derivatives $d(Gp)_{1-5}G$, dissolved in water, give rise to cholesteric and hexagonal mesophases. The results of X-ray Diffraction, Optical Microscopy, Circular Dichroism and Small Angle Neutron Scattering measurements indicate that the building block of the liquid-crystalline phases is a chiral rod, composed of a stacked array of Hoogsteen-bonded guanosine tetramers. The concentration at which the cholesteric phase appears increases with the oligomerisation degree, a pattern that seems to be related to the change of the ratio of negative charge/guanine units along the series.

Liquid crystalline structures in living organisms are not restricted to cell membranes. Hardening of the arteries is caused by deposition of liquid crystals of cholesterol esters on their walls. The cells involved in sickle cell anaemia have a liquid crystal structure[1]. Dinoflagellate chromosomes and the organic matrix of the cuticle of certain crabs display structures analogous to cholesteric liquid crystals[2]. In vitro experiments have shown that DNA and RNA solutions can spontaneously undergo a transition to a liquid crystalline state above a critical concentration, which essentially depends upon the length of the nucleic acid molecules[3]. In buffered solutions, calf thymus DNA fragments of about 146 base pairs give rise to a columnar hexagonal phase at concentrations > 300 mg/ml, whereas a cholesteric phase is formed at about 180-200 mg/ml[4]. Our finding[5] that, in special cases, lyotropic liquid crystals are formed at significantly lower concentrations by **sequences as short as a dinucleotide** stems from a fortuitous observation. One of us, while recording routine ^{1}H-NMR spectra of some dinucleoside monophosphates in D_2O, using Varian EM-390 spectrometer, obtained for a 5 % (w/w) solution of 2'-deoxyguanylyl-(3'-5')-2'-deoxyguanosine (**G 2**) the spectrum shown in Fig.1 (**top**). A very deceptive result indeed, since it features almost exclusively the resonance corresponding to H_2O, accompanied by intense side bands. If we had already been equipped with a better performing NMR spectrometer, we would have worked at much lower concentration and obtained a " correct " spectrum exactly like the one presented in Fig.1 (**bottom**), leaving the liquid crystalline properties of oligoguanylates for someone else to find. Instead, the lucky coincidence was that, a few days before, the person who recorded the spectrum had been warned by another author of the present paper, who was working on DNA liquid crystals, about possible "unusual" behaviour of synthetic oligomers. In fact, when observed with the appropriate techniques, the 5% solution of **G 2** displayed the fingerprint texture (Fig.2, left), which is characteristic of a cholesteric liquid crystalline phase. Peripheral evaporation showed that at higher concentration a transition occurs from the cholesteric to a hexagonal phase (Fig.2, right). The next step was to envisage a model structure for the building blocks of the mesophases, starting from available X-ray fiber diffraction data concerning the structure of gels formed by " sticky " guanosine and several of its monomeric derivatives[6]. In almost all cases, guanine bases are hydrogen-bonded in the **planar, tetrameric**

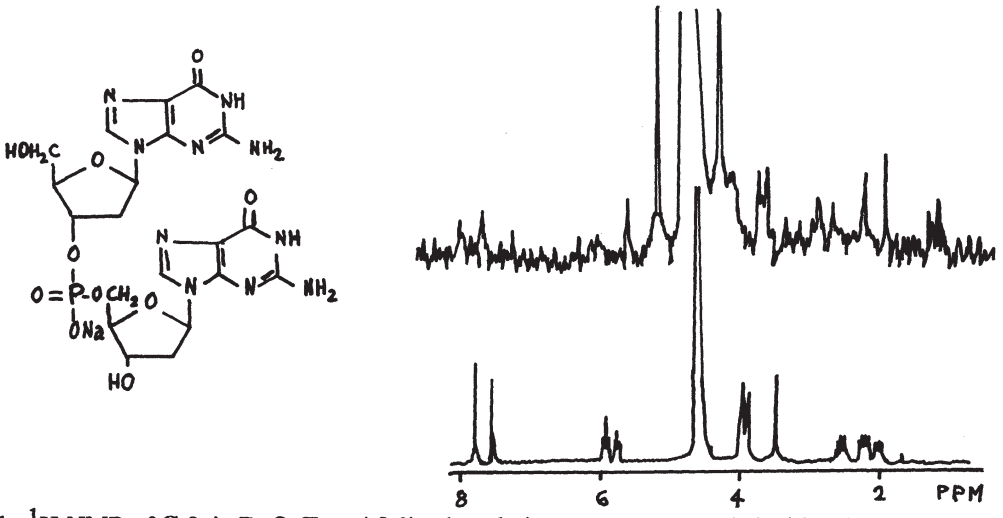

Fig.1. ^{1}H-NMR of G 2 in D_2O. Top: 4.8 % w/w solution, spectrum recorded with VARIAN EM-390. Bottom: 0.2 % solution, spectrum recorded with VARIAN VXR-200.

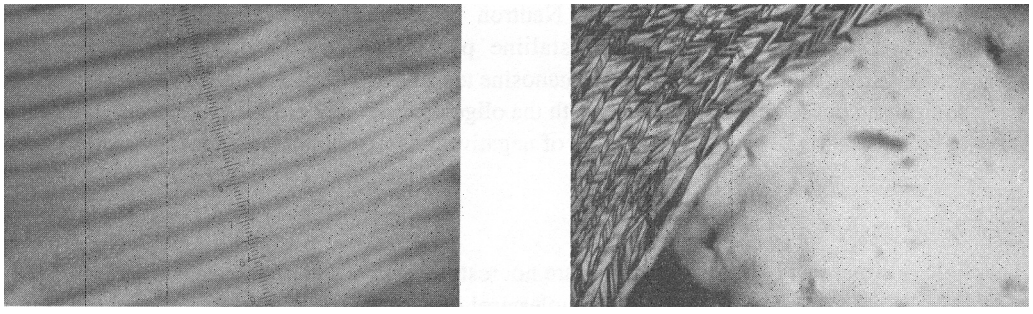

Fig.2. Liquid crystalline phases of G 2 in water (crossed polars, magnification 250 x). Left: fingerprint texture of the cholesteric phase, after magnetic alignement. Right: texture obtained by peripheral evaporation; the picture shows the transition between the cholesteric (lower right side) and the hexagonal (upper left side) phases.

arrangement shown in Fig.3 (Hoogsteen pairing) and further **piled on top of one another**, with sugar protruding at the periphery. The tetrameric layers do not stack in register, but are rotated with respect to each other; the extent and sense of rotation depend on the sugar moiety. Poly(G) gives a right-handed **quadruple helix** (pitch 39.4 Å, with the tetrameric planes equally spaced at 3.4 Å). A plausible model for the building blocks of the mesophases is a chiral rod of undefined length, similar to a four-stranded helix, composed of a

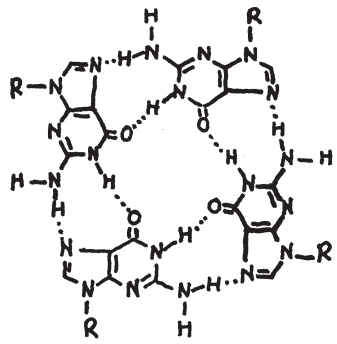

Fig.3. A tetramer of Hoogsteen-bondeed guanine residues.

stacked array of planar tetramers formed by four hydrogen-bonded guanosine moieties. This columnar aggregate can be formed by the dinucleoside monophosphate units in two different ways (Fig. 4). Either **discrete** pseudooctamers, formed by association of four dimers, are piled on top of each other as a result of stacking interactions between the tetrameric aromatic planes or, alternatively, the tetrameric layers, formed

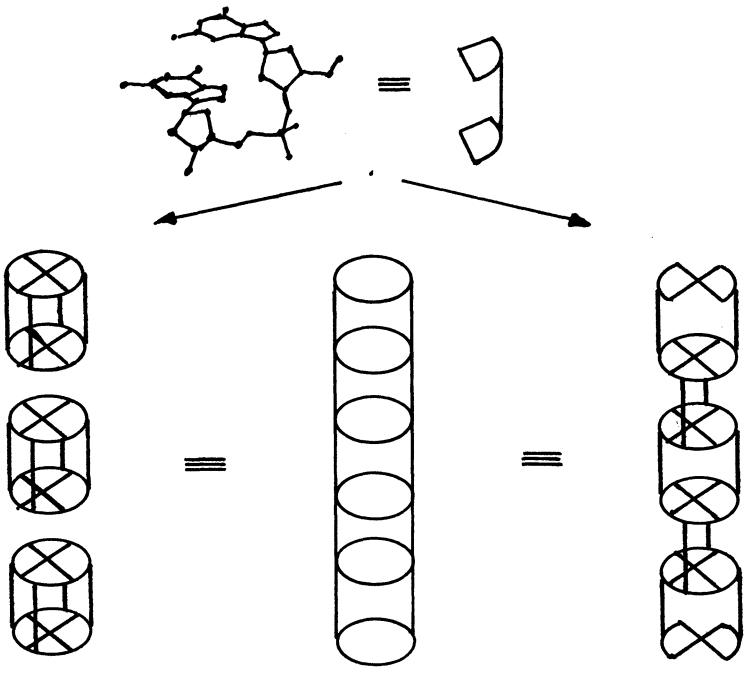

Fig.4. A sketch of two possible modes of formation of the rod-like aggregate from **G2** single molecules. Each circle (in perspective view) rapresents a planar guanine tetramer. For semplicity the helical distribution along the rod axis is omitted.

by staggered dimers, are held together by both covalent and non-covalent bonds in a **continuous** structure. The existence of a cholesteric liquid phase indicates that the cylindrical aggregates are chiral. These chiral rods are arranged in helicoidal superstructures in the cholesteric phase and side by side in the hexagonal one. Thus the deoxyguanosine dimer in water gives, at increasing concentration, the following phase sequence: isotropic (I), cholesteric (N^*), hexagonal (H) and crystal. Transitions from one phase to the other occur at a specific concentration value. The same behaviour was found with the other investigated oligoguanylates: the trimer d[$(Gp)_2G$] (**G 3**), the tetramer d[$(Gp)_3G$] (**G 4**) and the hexamer d[$(Gp)_5G$] (**G 6**) as well as with the monomer 2'-deoxyguanosine-5'-monophosphate (**G 1**). Each phase was studied by appropriate techniques[8]: **I** by circular dichroism (CD) and small angle neutron scattering (SANS), **N^*** by CD, optical microscopy (OM) and X-ray diffraction, **H** by X-ray diffraction. CD, being very sensitive to stereochemical changes is ideal for following the process of association from the isolated molecules to the supramolecular aggregate and the formation of the cholesteric phase. Furthermore, it allows the determination of the handedness of the

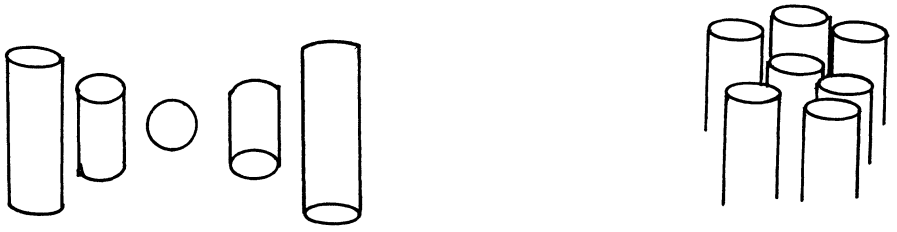

Fig.5. Arrangement of the chiral rods in the cholesteric (left) and hexagonal (right) mesophases.

cholesteric superhelix, whose pitch is measured directly by OM from the fingerprint texture of the magnetically aligned cholesteric. X-ray diffraction gives the spacing of the diffracting tetrameric planes within the rod, the rod diameter and the distance between the rods in the H phase. SANS data give information about the number, shape and dimensions of the scattering particles present in the isotropic phase. A detailed description of all the experimental results[8] is beyond the aim of this presentation, which is restrained to just some of the most relevant findings.

The phase sequence is reported below for all the derivatives, with the critical concentration expressed in weight of solute/ percent weight of solution.

G2	*I*	2.5	*N**	18	*H*
G3		8		25	
G4		13		35	
G6		19		35	
G1		30		40	

For derivatives **G 2 - G 6**, the critical concentration at which the cholesteric phase appears seems to follow a pattern related to the ratio of negative charge/guanine unit. This ratio is indicative of both the elecrostatic interactions and the hydrophilic/hydrophobic balance. The smaller this ratio, the easier the formation of the LC phases. For monomeric derivatives, which are structurally different from the oligomers, an investigation on several, differently substituted, compounds indicates that the hydrophilic / hydrophobic ratio is not the leading factor and that stereochemical features are very important.

For all derivatives, the X-ray diffraction data are in agreement with the proposed rod-like structure of the building units of the liquid crystalline phases, with the tetrameric planes equally spaced at a distance of about 3.3 Å. The rod diameter shows a dependence on the water content, while the oligomerisation degree has a very modest influence, if any.

The cholesteric is left-handed in the case of **G 1** and **G 2** and right-handed for **G 3, G 4** and **G 6**, with **G1** and **G6** giving the most compact superhelices. It is interesting that the cholesteric handedness and pitches can be correlated to the chirality features of the columnar aggregates.

The ability of oligodeoxyguanylates to give lyotropic liquid crystals can be viewed as the "extreme" consequence of the tendency of the guanine moiety to self-associate, a property that was first described in 1910[9]. Since stretches of contiguous guanosine are present in many chromosomal locations, their potential self-recognition and its possible biological role have been and are widely discussed and investigated.

Soon after our observations with the dinucleotide, D. Sen and W. Gilbert reported that synthetic single-stranded DNA fragments (20-49 bases long), containing two close homoguanylic tracts, associate at physiological salt concentrations into four-parallel-stranded structures. The result of competition experiments with the complementary strands led the authors to speculate that "self-recognition of guanine-rich motifs of DNA serves to bring together, and to zipper up in register, the four homologous chromatids during meiosis"[10].

Among the G-rich sequences present in DNA, the most widely studied are the telomeres, the ends of eukariotic linear chromosomes; they protect the chromosome from exonucleolytic degradation and end-to-end fusion. Telomeres consist of tandemly repeated sequences (from less than a hundred to thousands of base pairs) , with the G-rich strand oriented 5' to 3' toward the chromosomal terminus and protruding 12-16 nucleotides beyond the complementary cytidine-rich strand. The structures of these single-stranded overhangs have been probed by model studies with synthetic oligonucleotides.

Two such studies have very recently appeared in the literature, both concerned with the sequence $d(G_4T_4G_4)$, the terminus of the Oxytricha chromosomes. The structures that have emerged from single crystal X-ray diffraction (A)[11] and ^{1}H-NMR (B)[12] are both symmetrical, **bimolecular**, four-stranded helices, with four G-quartet planes (Fig. 6). However, while **A** is an **edge-looped structure**, which can be formed by dimerisation of two separate hair-pins, the **diagonally looped structure B**, found in solution, cannot result from direct joining of two preformed hairpins, since the guanines of one strand are hydrogen-

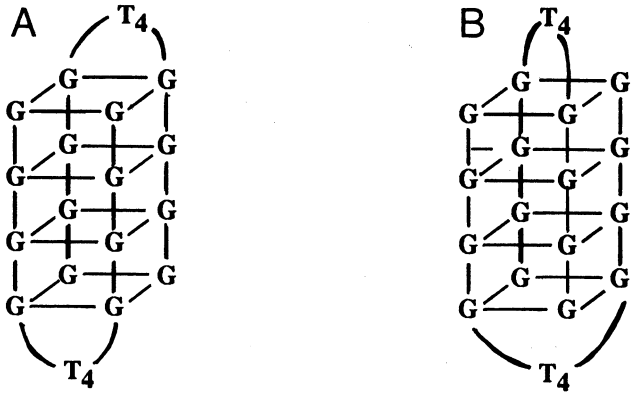

Fig.6. Sketches of the crystal (**A**) and solution (**B**) structure of $d(G_4T_4G_4)$.

bonded only to guanines of the other strand. It appears that different quadruplexes can be formed by the same sequence, depending upon different ambient conditions, namely, in the case at hand, the nature of the cations present in solution.

This finding leads to one of the main, open questions of our research: the factors playing the major roles in the making and stability of the chiral rods which constitute the liquid crystalline phases. Meaningful information on the aggregation process can be obtained from the isotropic phase, where, depending on the concentration, only single molecules are present on the one extreme, and still uncorrelated rods on the other.

Small angle neutron scattering data, recorded at 1% w/w concentration, show for all oligoguanylates but **G 1** the presence of cylindrical particles having a length of 60-70 Å and a diameter of about 25 Å. This last value is in agreement with the X-ray diffraction data obtained from the liquid crystalline phases. The percent of aggregation spans from 33 for the tetramer to 44 for the dimer, but these values are affected by a large experimental error.

As already mentioned, circular dichroism, because of its sensitivity to stereochemical variations, is ideal for following the process of association from the isolated molecules to supramolecular aggregates and finally to the cholesteric. Fig. 7 presents the concentration dependence of the CD spectrum of the **sodium** salt of **G 2** in water (left), and the temperature dependance of a very dilute solution of the same salt in a **potassium** buffer (right). At 0.003% concentration, the spectrum of the sodium salt in water (left, trace 4) is that of the isolated **G 2** molecules, whereas in the presence of potassium ions (right, trace 1) it closely resembles that of the totally aggregated state of the dimer (left, trace 1)[8] . This last spectrum is not very different from that of the four-stranded helix of poly(G)[13]. Thus, the specific cation effects can be monitored by CD technique and confirm the stabilizing role of potassium for aggregates formed by G-rich sequences. The thermal behaviour is also very informative, since it can be related directly to the stability of the aggregates formed by

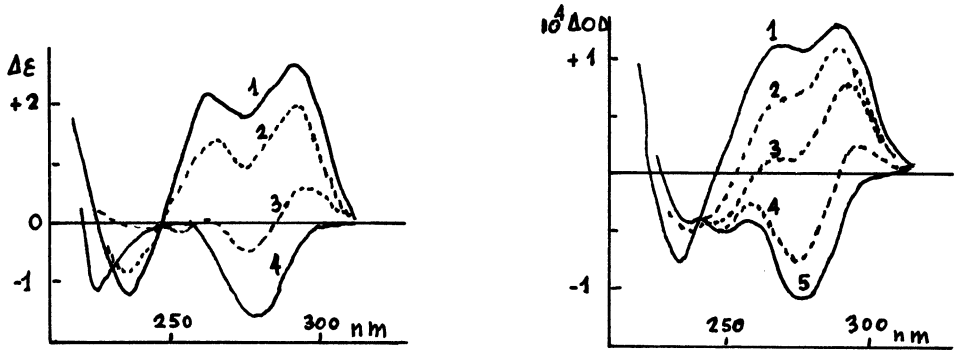

Fig.7. CD spectra of **G 2**. Left: concentration (%, w/w) dependence in water; c = 2.3 (1), 1.5 (2), 1.04 (3), 0.003 (4). Right: temperature dependence of a 0.003 % solution in potassium phosphate buffer(pH 7); T = 20 °C (1), 40 °C (2), 45 °C (3), 50 °C (4), 60 °C (5).

different oligoguanylates. For instance, under the same experimental conditions, the higher aggregated form of **G 2** melts at about 40°C, while that of **G 3** melts at about 80° C. This finding suggests that the stability of the rod-like aggregate directly correlates with the number of phosphate bridges connecting the tetrameric G-planes.

So far, two general types of molecules have been considered: G-rich sequences, which give aggregates containing a maximum of four single units, and short homo-G sequences, that form higher order aggregates (the rods), which self-assemble into liquid crystal structures. Very recent results show that also G-rich oligomers may give higher order aggregation, depending on the location of the extra base(s) within the sequence. D. Sen and W. Gilbert reported[14] that, while d(T_8G_3T) forms only one type of aggregate containing 4 strands , the isomeric d(T_9G_3) gives stable aggregates made of 8, 12 and 16 distinct strands. Moreover, a cholesteric phase is formed by the sodium salt of d(G_2AG_2) at a concentration of about 15 % (w/w)[15].
This last result shows that the ability to give liquid crystals is not restricted to homo-G oligonucleotides, but can be desplayed also by properly tailored G-rich sequences.

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