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A study of the self-assembly of 2′-deoxyguanosine 3′:5′-cyclic monophosphate, d(cGp), by CD and X-ray diffraction

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2′-Deoxyguanosine 3′:5′-cyclic monophosphate forms in water cholesteric and hexagonal columnar mesophases. The polymorphic behaviour and the structural building blocks of the liquid crystalline phases, as determined by optical microscopy, CD spectroscopy and X-ray diffraction, are comparable to those found with all the deoxyguanylates investigated so far (in particular with deoxuguanosine 5′- and 3′-monophosphate). The present results show that the formation of a stacked array of planar G-tetramers, a necessary condition for the existence of the columnar mesophases, occurs even in the absence of hydrogen bonding groups linking the molecules along the length of the columns.

1. Introduction

Guanosine, 2′-deoxyguanosine and their derivatives give gels in water [1]. The first report on this phenomenon appeared in 1910 [2], but the explanation of this peculiar behaviour came fifty years later, from an X-ray diffraction study of fibres obtained by drying the gels formed at pH 5 by guanosine 5′- and 3′-monophosphate [3]. Both compounds form very similar, highly ordered helical structures, characterized by the presence of guanine bases hydrogen bonded in the tetrameric arrangement shown in figure 1. The helix formed by guanosine 3′-phosphate consists of a core of equally spaced, planar G-tetramers, with the sugar phosphate moieties protruding at the periphery. Later on, studies by X-ray fibre diffraction and model building indicated that poly(G) forms a right-handed, quadruple helix, where the planar G-tetramers are connected by the four covalent sugar-phosphate backbones [4]. Several nuclear magnetic resonance investigations of aqueous solutions of guanosine 5′-monophosphate at neutral pH demonstrated that, by a concentration dependent process, the compound gives first planar tetramers that, by stacking on top of one another, form octamers and dodecamers [5]. The distribution and stability of the various aggregates were found to depend strongly upon the nature and concentration of added alkali metal ions [6]. Model building studies led the authors to suggest that interplanar hydrogen bonds, involving the phosphates groups, might also contribute to the stability of the

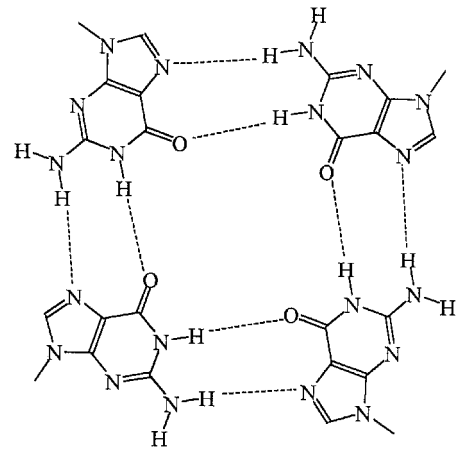

Figure 1. The tetrameric arrangement of guanine bases.

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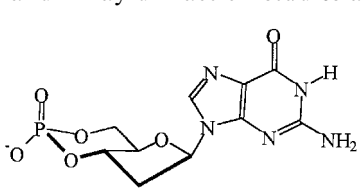
multilayered aggregates [6]. The ordering ability of guanine is not restricted to homoguanylic mono- and poly-nucleotides. Short guanine-rich oligonucleotides, made of a few repeats of telomeric DNAs, are able to self-assemble into monomeric, dimeric and tetrameric structures, held together by the planar G-tetramers. Several such structures, and the processes leading to them, have been elucidated by CD [7] and NMR [8] spectroscopies and single crystal X-ray diffraction [9], but a clear understanding of the role of guanine self-association in telomer function has yet to emerge.

That guanine-driven assembly may result in ordering beyond the quadruple helix was first shown by optical microscopy using aqueous solutions of the sodium salt of 2′-deoxyguanylyl-(3′-5′)-2′-deoxyguanosine, d(GpG), that forms cholesteric and hexagonal mesophases [10]. The same behaviour was displayed by the other homoguanylates investigated. The concentration at which the cholesteric phase appears increases with the degree of oligomerization and seems to be related to the ratio of negative charge/guanine units in the molecule [11]. Further studies, including X-ray diffraction, proved that the building block of the liquid crystalline phases is a chiral rod, with a diameter of about 25 Å, composed of stacked guanine tetramers [11]. Furthermore, small angle neutron scattering (SANS) data recorded for all compounds at 1% w/w concentration (isotropic phase) showed the presence of cylindrical particles with a length of 60–70 Å and a diameter of about 25 Å [12]. Liquid crystalline phases are formed, too, by the ammonium salts of 2′-deoxyguanosine 5′- and 3′-monophosphate [d(pG) and d(Gp), respectively], but the appearance of the cholesteric mesophase occurs at very different concentrations (30 and 5% w/w, respectively [13]). Also, SANS failed to reveal the presence of cylindrical particles in the 1% w/w solution of d(pG) [12]. CD studies of isotropic solutions of the two homoguanylates in the absence and in the presence of KCl, showed that d(Gp) forms aggregated species at lower concentration than d(pG) [13].

Since all available evidence indicates that the free monophosphate group and its location may play a major role in the self-assembly of the monoguanylates, we have examined the behaviour of 2′-deoxyguanosine 3′:5′-cyclic monophosphate d(cGp), where hydrogen bonding groups 'pendant' from the sugar ring are no longer present. The results of optical microscopy, CD spectroscopy and X-ray diffraction studies are reported.



d(cGp)

2. Results and discussion

2.1. *Optical microscopic observations*

The phase sequences shown at room temperature by sodium and ammonium salts of 2′-deoxyguanosine 3′:5′-cyclic monophosphate, as determined by observation of the typical textures exhibited by the different phases in polarized light (and then confirmed by X-ray diffraction), are the following:

ammonium salt I-6·5%-Ch-16%-H
sodium salt I-20%-Ch-35%-H

where I, Ch and H represent the isotropic, cholesteric and hexagonal phase, respectively.

Concerning the cholesteric phase, we could obtain neither the fingerprint texture nor the planar texture even after the sample had been kept in a 5 kG magnetic field for a long time. This finding, in contrast to what is observed for many guanosine derivatives investigated [11], has been already reported for d(Gp) and its isobutyl ester [14]. Considering that the guanine tetrameric planes tend to align parallel to the magnetic field, this behaviour could indicate that the guanine residues which form the tetramer are not exactly perpendicular to the axis of the aggregate. Therefore the columnar aggregate could have a small diamagnetic anisotropy or, perhaps, the columns are too entangled.

2.2. *CD experiments: the assembly process from the isotropic solution to the cholesteric phase*

Circular dichroism spectroscopy is very sensitive to stereochemical variation; it is ideal for following the aggregation process of d(cGp) from the isolated molecules to the supramolecular aggregates and finally to the cholesteric mesophases. CD spectra of aqueous solutions of sodium and ammonium salts of d(cGp) at different concentrations, with or without added 1M KCl, have been recorded at different temperatures, and with careful repetition of the thermal cycles.

Typical CD spectra of solutions of d(cGp) are reported in figures 2–4. The main features of these spectra are quite similar to those already reported [13, 14] for the deoxynucleotides d(pG), d(Gp) and its isobutyl ester. From an analysis of these figures, three characteristic spectra can be identified.

(a) The spectrum of the isolated monomeric species can be observed in the more dilute solutions in pure water; the same spectrum is also obtained at higher temperature for more concentrated solutions or in the presence of salts. They show weakly dichroic signals at *c.* 280 and 250 nm ($\Delta\varepsilon$ *c.*+0·3 and −0·1, respectively), followed by a positive ($\Delta\varepsilon$ *c.*+3) signal at *c.* 210 nm.

(b) The spectra of the assembled species can be observed at intermediate temperatures and concentrations of d(cGp); they are drastically different from the spectrum of the monomer and are characterized by a relatively intense, non-conservative, negative, exciton-

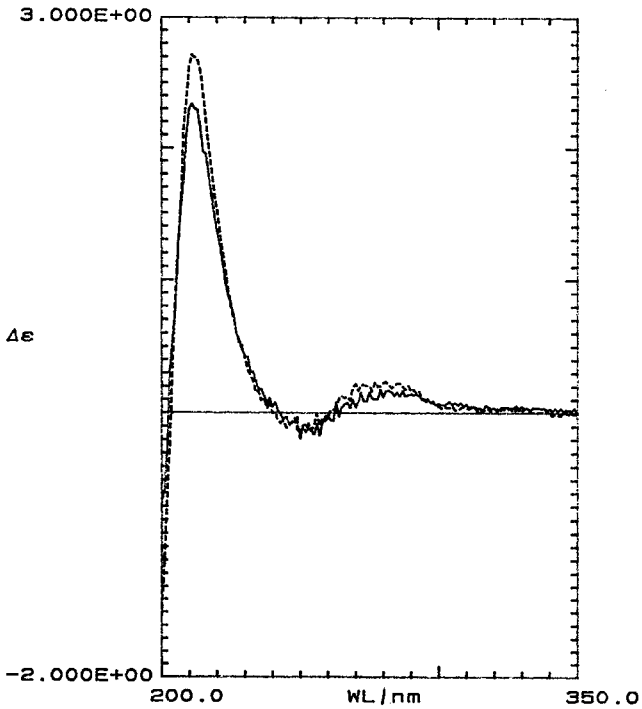


Figure 2. CD spectrum of the sodium salt of d(cGp), 0·4% w/w in water, recorded at 5° (dotted) and 80°C (solid line).

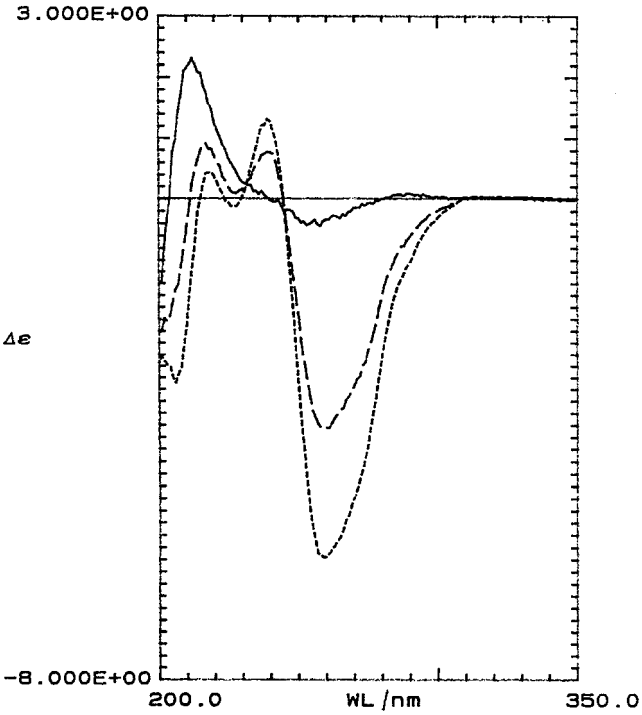


Figure 3. CD spectra of the sodium salt of d(cGp), 0·4% w/w in KCl 1M, recorded at 0° (dotted), 10° (dashed) and 20°C (solid line).

like couplet [15] centred around 245 nm. The amplitude of the couplet ($\Delta\Delta\varepsilon$ *c.*−7) depends on the concentration of the guanine derivatives and on the salts added.

(c) At much higher concentrations (or lower temperatures), an enormous negative band ($\Delta\varepsilon$ *c.*−70) is observed; this spectrum, whose shape is similar to that of the absorption spectrum, is associated with the formation of the cholesteric mesophase.

The CD spectra of the aggregate can be interpreted in the light of previous results. Firstly, the CD spectra of the four-stranded helices of poly(G) and poly(dG) are known, and also the structure of this unusual helix, which is similar to our columnar aggregates, is known in detail from fibre X-ray work on poly(G): the helix is right-handed and the bases are stacked perpendicularly to the helix axis [4]. Secondly, the spectroscopic properties of guanine have been extensively investigated and, consequently, it is possible to calculate with a high degree of confidence the CD spectra of poly(G) and of similar molecules with a relatively simple exciton treatment [16]. From this approach, the chirality of the columnar aggregates can be deduced. In the four-stranded helix of poly(G), the transition at *c.* 250 nm gives rise to a non-symmetric exciton couplet with a stronger positive component at *c.* 260 nm and a weaker negative band at *c.* 240 nm. It follows that, whenever spectra similar to this are obtained, they can be related to the presence of right-handed four-stranded structures. The correlation has both empirical and theoretical validity.

For the derivative d(cGp), the aggregates have a CD spectrum almost mirroring that of poly(G); thus a left-handed columnar helicity can be inferred.

The interpretation of the intense signal due to the cholesteric order can be obtained in the light of Mauguin's model extended to the absorption region [17]; this also allows the determination of the cholesteric handedness if a few spectroscopic characteristics of the structural unit forming the mesophase are known. In the case of a stacked system of guanine tetramers, a positive CD signal in the absorption region indicates a right-handed cholesteric, and a negative CD signal indicates a left-handed cholesteric. For d(cGp), the cholesteric phase is left-handed (*M*).

In summary, from CD experiments it can be deduced that d(cGp) dissolved in water, with or without the presence of KCl, gives rise to a self-recognition and self-assembly process leading to left-handed columnar aggregates and finally to left-handed cholesteric mesophases; the presence of an excess of K^+ helps the aggregation process.

2.3. *X-ray diffraction experiments: the identification of the mesophases and the phase diagram*

The structure of the liquid crystalline phases (in particular of the hexagonal phase) formed by the sodium

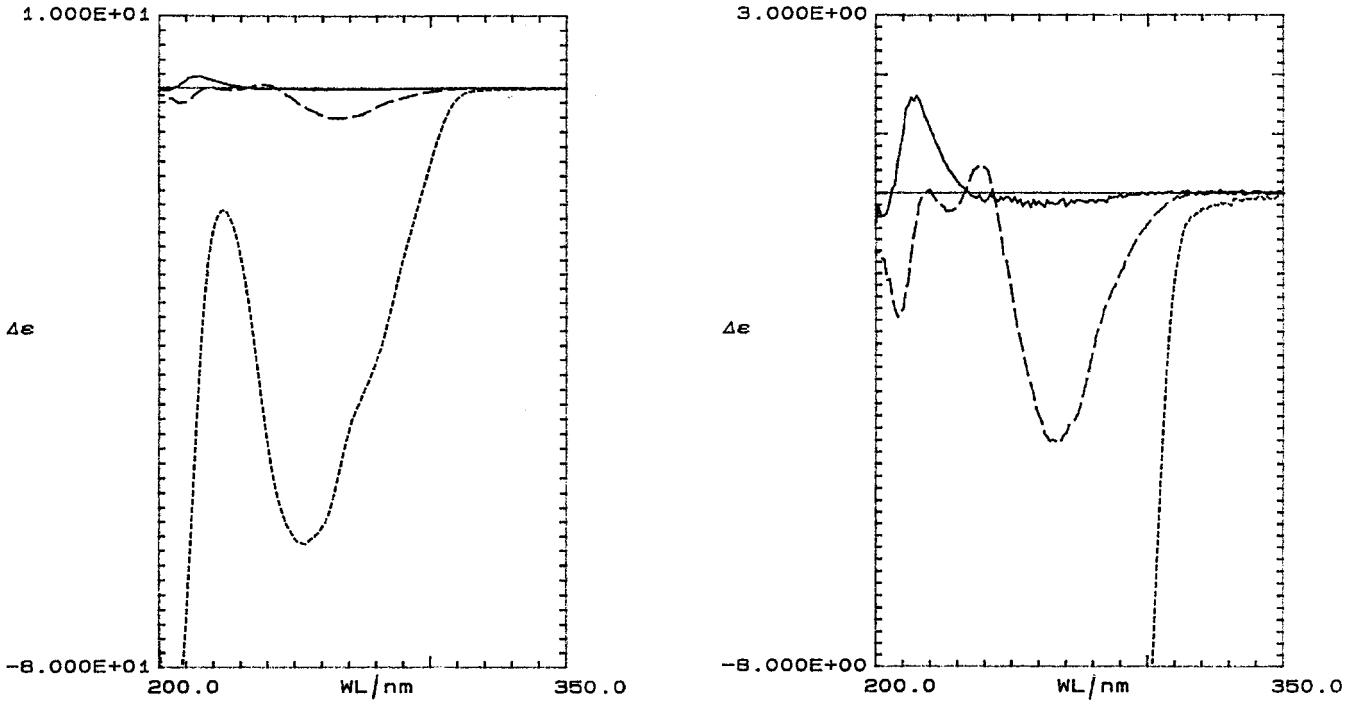


Figure 4. CD spectra of the sodium salt of d(cGp), 4% w/w in KCl 1M, recorded at 30° (dotted), 50° (dashed) and 55°C (solid line).

salt of d(cGp) can be derived from the analysis of the corresponding X-ray diffraction profiles. Typical X-ray diffraction patterns for the two mesophases observed in the present system are reported in figure 5.

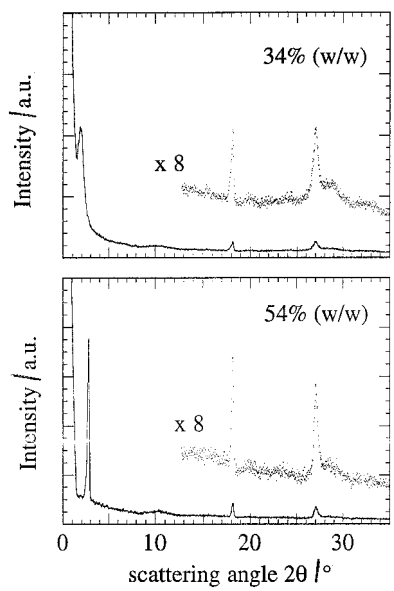


Figure 5. X-ray diffraction patterns obtained at 25°C from d(cGp) samples with $c=54\%$ (upper frame) and $c=34\%$ (lower frame). The peaks centred at 2θ of $c.$ 18° are due to the mica windows.

The narrow band observed in the high angle region at a reciprocal spacing s of about $(3{\cdot}4\,\text{Å})^{-1}$ ($s=2\sin\theta/\lambda$, where 2θ is the scattering angle and λ the X-ray wavelength) is indicative of columnar liquid crystalline phases [10, 11, 14, 18]. This reflection is present in both mesophases: in fact, it is related to the intracolumnar order of the structural elements [19]—the thickness of any aromatic ring—composed of a stacked array of guanine tetramers at the van der Waals distance.

In the low-concentration mesophase only a broad peak is observed in the low angle region, as expected for a cholesteric phase. In the high concentration mesophase, up to five peaks are easily identified in the low angle region. The peak positions are in the ratio $1:3^{1/2}:4^{1/2}:7^{1/2}:\ \ldots$ and are indicative of a two-dimensional hexagonal lattice of *p6m* symmetry [20, 21]. It should be noted that no extra peaks were observed in the low angle region; this indicates that in the mesophase no long range intercolumnar correlation of the tetramer units exists. In other words, there is no longitudinal positional order, and the rods may freely translate in the direction perpendicular to the two-dimensional hexagonal cell [22].

The phase diagram obtained from X-ray diffraction experiments performed at different temperatures and concentrations is reported in figure 6. It is interesting to note that the cholesteric phase disappears at high temperature. In its general appearance, this phase diagram

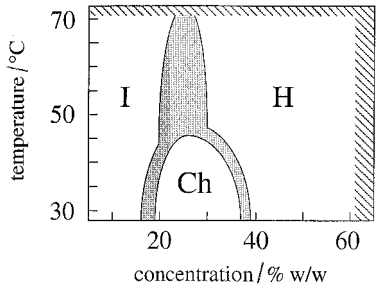


Figure 6. Phase diagram of d(cGp) as deduced from X-ray diffraction measurements. I: isotropic; Ch: cholesteric; H: hexagonal phases. Shadowing indicates regions of phase coexistence. Hatched areas were not investigated.

is in agreement with the diagram determined for d(pG) [18], as well as with phase diagrams calculated for self-assembling rod systems [23, 24]. These theoretical approaches indicate that the stability of a columnar cholesteric phase depends on the properties of the aggregate (such as flexibility and length) and on the aggregation strength; only in the case of strong aggregation (*e.g.* for a long and/or stiff aggregate) may a stable nematic (cholesteric) phase be observed. This seems to be the situation in the present system; the nematic phase disappears on increasing the temperature as a consequence of the reduced aggregation strength (and stiffness or length).

2.4. *X-ray diffraction experiments: the structural properties of the columns*

From the analysis of the position and shape of the diffraction peaks, information about the properties of the hexagonal phase and of the structural elements from which it is formed could be obtained. In figure 7, the variation of the unit cell dimension as a function of concentration and temperature is reported. As expected,

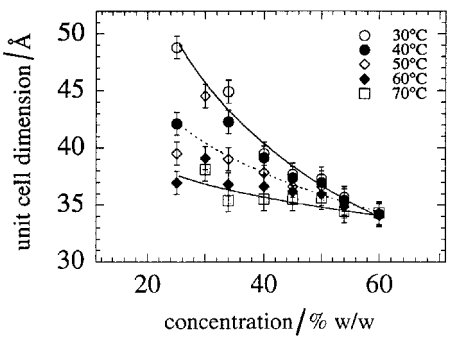


Figure 7. Interaxial distance between four-stranded helices as a function of concentration of d(cGp) at different temperatures. The lines represent fits to the data obtained at 30°, 40°, 50°, 60° and 70°C using a power law; the resulting exponent was equal to −0·34 (correlation coefficient R= 0·99), −0·20 (R=0·82) and −0·10 (R=0·82).

we found a monotonic decrease of the unit cell dimension with increasing concentration†. Moreover, a significant thermal effect was detected: while at higher concentrations data appear to be fairly independent of temperature, at low concentrations, interaxial distances show a strong inverse temperature dependence.

For flexible rod systems, theoretical calculations [23, 24, 25] showed that the exponent of the power law dependence of a with c_v† varies from $-1/2$ to $-1/3$ as a function of flexibility ($-1/2$ being for spherocylinders that are flexible, longer than the persistence length, and $-1/3$ for rigid spherocylinders, shorter than the persistence length). Power law behaviours were then calculated from the curves reported in Figure 7: at 30°C, the data align on the curve with $-1/3$ exponent, but at higher temperatures, the data vary more slowly than $c^{-1/3}$. The relevance of those results for the derivation of a model for the hexagonal phase will be discussed in the next paragraph.

As reported in [18], the tetramer stacking repeat distance and the average number of stacked discs per column could be obtained by analysing the position and the full-width and half-maximum of the high angle $(3{\cdot}4\,\text{Å})^{-1}$ peak. The results are summarized in figures 8 and 9. In figure 8, the tetramer stacking distance as a function of concentration and temperature is reported. In spite of the considerable scatter in the data, the general trend is for a small increase of the repeat distance

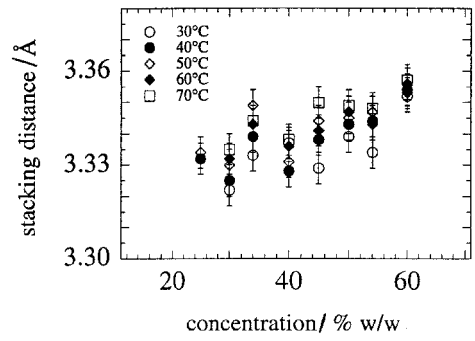


Figure 8. Distance between stacked tetramers as a function of concentration of d(cGp) for different temperatures.

† Let us assume that columns have a circular section with radius R and stress that the radius of the tetramer is expected not to change as a function of concentration. If columns in the hexagonal phase are rigid and infinitely long, the relation between the cross-sectional area of the cylinder and the two-dimensional hexagonal unit cell surface is [10, 20]: $\pi R^2 = (3^{1/2}/2)a^2c_v$. Therefore, the rods move apart only laterally as dilution proceeds and a will change with concentration as $c_v^{-1/2}$. By contrast, if the structure is composed of cylinders of fixed length L, packed as a fluid in the direction normal to the hexagonal plane and with an average distance C between rod centres, we obtain [18]: $L\pi R^2 = C(3^{1/2}/2)a^2c_v$. In this case the dilution will increase both C and a dimensions.

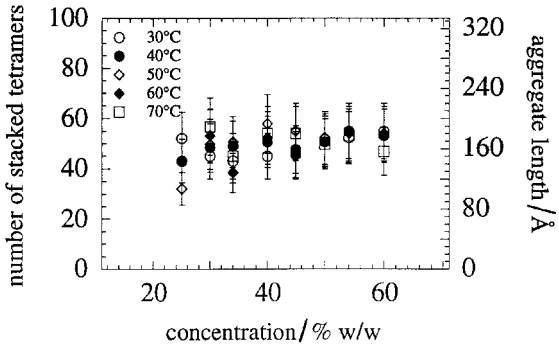


Figure 9. Average number of stacked tetramers, determined as reported in [18], as a function of concentration of d(cGp) at different temperatures. In the right hand scale, data are converted into average aggregate lengths.

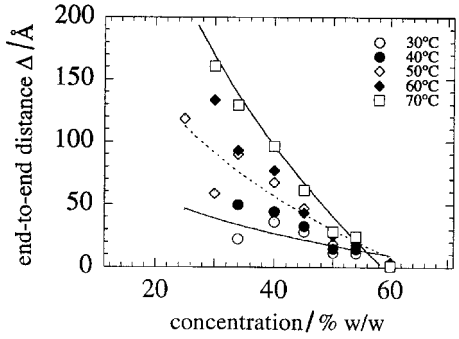


Figure 10. Intercolumnar end-to-end distance Δ between the four-stranded helices in the hexagonal phase as a function of concentration of d(cGp) for different temperatures (the absolute error is estimated to be ± 20 Å). The lines are power fits to the data obtained at 30°, 50° and 70°C to show the general trend.

with increasing temperature and concentration. Noticeably, the stacking distance is lower than the measured values for d(pG) and d(Gp), which might indicate that the electrostatic repulsive interactions between the discs are reduced. The average length of the columnar aggregates is reported as a function of concentration and temperature in figure 9. The length of the aggregate, *c.* 160 Å, appears independent of temperature and concentration. The absence of any growth in length of the columns as a function of concentration, as observed for d(pG) and folic acid [18, 26], confirms that, in the present case, the aggregation of the tetramer is strong.

2.5. *A model for the phase diagram*

From the analysis of the X-ray diffraction profiles and simple geometrical considerations, an interpretation of the phase diagram may be given. The hexagonal phase may be considered to be formed by columns of stacked discs of radius R and length L, with an end-to-end distance between two columnar aggregates Δ. The Δ values may be calculated using the following equation [18]:

$$\Delta = L(2\pi R^2/c_v a^2 3^{1/2} - 1)$$

The distances calculated from the data in figures 6 and 8, using $R = 12{\cdot}5$ Å, are reported in figure 10. The behaviour is similar to that reported for d(pG) [18]: at low temperature, the addition of water mainly increases the lateral distances between the cylinders (while the end-to-end separation only smoothly increases); as dilution proceeds the lattice parameter increases until the hexagonal packing becomes unstable relative to the cholesteric phase. At high temperature, dilution mainly determines an increase of the end-to-end distance between columns and the increase of a is much slower; a direct transition to the isotropic phase (without a cholesteric phase) occurs, when the intercolumnar end-to-end separation becomes high (up to 60% of the aggregate length) as a consequence of a very loose packing in the C direction‡.

3. Conclusion

Several deoxyguanylates, including 2′-deoxyguanosine 3′- and 5′-monophosphates, form lyotropic liquid crystals. As established by X-ray diffraction studies, the common building blocks of the mesophases are columnar aggregates, already present in the isotropic solution, whose core is a stacked array of planar G-tetramers. In the rods formed by oligoguanylates, the tetramer planes are held together primarily by covalent sugar phosphate

‡These results offer further support for the suggestion of a dominant role of water in modulating the columnar polymorphism observed in guanosine derivatives. In fact, short range hydration forces have been recognized as responsible for the formation of the condensed phase in several linear macromolecular aggregates such as DNA, polysaccharides and collagen [27], and also in d(pG) four-stranded helices [28]. Hydration forces can be either strongly repulsive or attractive; attractive forces are accompanied by the release of structured water into the bulk, an effect which has been recognised as the most probable source of increased entropy after assembly [29, 30]. Moreover, attraction strengthens with increasing temperature [30]. Data obtained in this work appear consistent with a balance of attractive and repulsive forces between the d(cGp) columns. At the lower temperatures, the repulsive hydration (and electrostatic) force dominates the interaction, and then the addition of water mainly increases the lateral distance between the cylinders.

Because of the presence of attractive hydration contributions induced and/or strengthened by temperature, the helices remain closer at high temperature as dilution proceeds. Water release from the two-dimensional hexagonal unit cell accompanies this effect; the increase in the end-to-end distance suggests that water is mainly localized between the cylinders in the axial region. Consistently, the temperature effect is concentration dependent, as the effectiveness in the release of structured water requires sufficiently hydrated samples.

bridges, whose number increases with the degree of oligomerization. An important role is also played by cations like K^+, whose size fits perfectly into the central cavity between adjacent tetramers. In the case of monoguanylates, several pieces of evidence indicate that the free phosphate group and its location play a leading role in the overall stability of the columns and their self-assembly, most likely by forming hydrogen bonds between the sugar phosphate moieties of adjacent layers. Thus, we were curious to see if cyclic 3′:5′-deoxyguanosine monophosphate, whose structure does not allow the formation of this type of interplanar hydrogen bonding, would still self-associate into chiral rods, ultimately forming liquid crystalline mesophases. In fact, the present results show that d(cGp) forms cholesteric and hexagonal phases at critical concentrations which depend strongly on the nature of the cation; ammonium ion promotes the assembly process far better than sodium, as already found for the acyclic monophosphates. The transition from the isotropic to the cholesteric phase occurs at a concentration (6·5% w/w) close to that found for the 3′-monophosphate derivatives d(Gp) (5%) and much lower than the corresponding value (30%) for the 5′-monophosphate derivatives d(pG). Also, the phase sequence displayed by d(cGp) is the same as that given by d(pG), while for d(Gp) four different mesophases are formed. X-ray diffraction studies show that the building block of the liquid crystalline phases formed by the cyclic monoguanylates is a columnar aggregate made of stacked guanosine tetramers, whose spacing, 3·4 Å, and radius, 12·5 Å at 50% w/w, are comparable to those found with all the deoxyguanylates investigated up to now. Also the packing of the cylindrical aggregates in the hexagonal phase is maintained throughout the series, as shown by the value of the unit cell dimension found with the cyclic derivative. CD spectra show that both the handedness of the aggregate formed in the isotropic phase and that of the cholesteric superhelix is the same for the three monoguanylate derivatives. The thermal stability of their aggregates in the isotropic phase is consistently low and its dependency upon the nature and concentration of added salt is comparable. For all three monoguanylates, the nature of the counterion affects strongly the stability of the cholesteric phase, which for the acyclic monophosphates is formed by the ammonium, but not by the sodium salt. Altogether, the present results show that the formation of a stacked array of planar G-tetramers, which is a necessary condition for the existence of the columnar mesophases, may occur even in the absence of covalent phosphate bridges or hydrogen bonding groups to connect the layers. Quite obviously, in this case the self-assembly process depends strongly upon the presence of cations like ammonium and potassium which, by coordination with the keto group of guanines, can stabilize the interaction between adjacent tetramer planes.

4. Experimental

4.1. *Materials*

The compound d(cGp) is a commercial product from Sigma.

4.2. *CD experiments*

CD spectra were recorded with a JASCO J710 spectropolarimeter equipped with a Neslab RTE-111 circulator thermostat (thermal stability $<1°C$) and using 0·001–0·1 cm cells with a thermostatting jacket. Solutions were prepared by dissolving the compounds (0·04–4% w/w) in water or in an aqueous solution of KCl ($1\,mol\,l^{-1}$). The solutions were allowed to stand at ambient temperature for one day before recording the spectra; also before recording the spectra after the first thermal cycle, the solutions were allowed to stand at ambient temperature for one day (the spectra did not change after the longer time). Concentrations of d(cGp) were determined spectrophotometrically at 80°C (λ 252 nm, ε 13700) and are expressed in % w/w. Noise reduction was obtained by accumulating several spectra (8–16) and by adopting a mathematical smoothing routine (JASCO software).

4.3. *Optical microscopy*

Microscopic observations were carried out with a Zeiss polarizing microscope equipped with a photocamera. Preliminary observations were made on samples with peripheral evaporation. Cholesteric solutions were inserted into rectangular capillaries (thickness 0·3 mm, from Vitrodynamics) and sealed with wax; the samples were then put into a 0·5 T magnetic field for at least 5 h in order to establish their behaviour.

4.4. *X-ray diffraction*

The liquid crystalline solutions were left for at least two days at room temperature to avoid inhomogeneity. The homogeneity of the samples was verified by optical polarizing microscopy. The relative uncertainty in the concentrations was estimated to be 5%. Concentrations are reported as weight/weight (c) or volume concentration (c_v), as appropriate. The volume concentration was calculated using for d(cGp) the specific volume $0{\cdot}651\,cm^3\,g^{-1}$. Low-angle X-ray diffraction experiments were performed using a 1·5 kW Ital-Structures X-ray generator equipped with a Guinier-type focusing camera operating in vacuum: a bent quartz crystal monochromator was used to select the Cu-Kα_1 radiation ($\lambda = 1{\cdot}54$ Å). The samples were mounted in vacuum-tight cells with thin mica windows. In order to reduce the spottiness arising from possible macroscopic monodomains, the

cells were continuously rotated during the exposure. The sample cell temperature was controlled with an accuracy of 1°C by using a circulating thermostat. The diffraction patterns were recorded on a stack of four Kodak DEF-392 films. Scattering data were also recorded on a two-circle diffractometer equipped with a bent position sensitive detector (INEL CPS120). A Philips PW1830 was used as X-ray source, run at a power of 1·6 kW with a copper target. The CuKα_1 line was selected by a monochromator focused on the detector. The intrinsic resolution of the diffractometer (width of a crystalline peak) was determined to be 1·1 channels. The width of one channel was measured using a LiF single crystal and calculated to be 0·0300(2)°.

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