Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713926090

The self-assembly and lyotropic mesomorphism of riboguanylic acids (GMP)

Giovanni Gottarelli; Gloria Proni; Gian Piero Spada

Online publication date: 06 August 2010

To cite this Article Gottarelli, Giovanni , Proni, Gloria and Spada, Gian Piero(1997) 'The self-assembly and lyotropic mesomorphism of riboguanylic acids (GMP)', Liquid Crystals, 22: 5, 563 — 566
To link to this Article: DOI: 10.1080/026782997208956
URL: http://dx.doi.org/10.1080/026782997208956

LIQUID CRYSTALS, 1997, VOL. 22, NO. 5, 563–566

The self-assembly and lyotropic mesomorphism of riboguanylic acids (GMP)

by GIOVANNI GOTTARELLI, GLORIA PRONI and GIAN PIERO SPADA*

Dipartimento di Chimica Organica 'A. Mangini', Università di Bologna,
Via S. Donato 15, 40127 Bologna, Italy

(*Received 30 November 1996; accepted 6 January 1997*)

The three isomeric guanylic acids (5′-GMP, 3′-GMP and 2′-GMP) and the 3′:5′-cyclic derivative (*c*GMP) form, in water solution, left-handed columnar aggregates which, at higher concentration, pack to give left-handed cholesteric mesophases. The self-assembly process and liquid crystal formation are discussed in relation to the behaviour of the 2′-deoxy analogues.

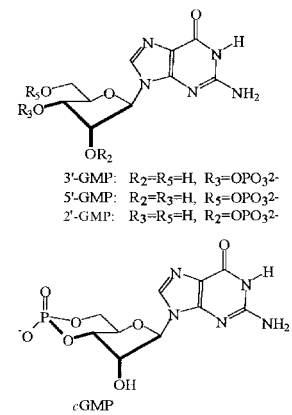
1. Introduction

In recent years there has been a revival of studies on guanine-rich oligonucleotides, mainly because of their biological significance [1]. The peculiar ability of guanosine and of some of its derivatives to self-associate to give stable structures has been known for a long time [2, 3]: crystals [4], fibres [5], highly ordered gels [6], and chiral aggregates [7] have been described.

More recently, we reported the formation of columnar chromonic [8] mesophases from several deoxyguanosine derivatives [9–12]. The common basic building blocks of these lyomesophases are chiral columnar aggregates, already present in isotropic solutions [13, 14]; their inner part is composed of a stacked array of planar units, the G-quartets, formed by four guanines, hydrogen-bonded according to a Hoogsteen mode (figure 1), while the external part is composed of hydrophilic sugar phosphate groups. Because of the dissymmetric nature of the molecules, the G-quartets do not stack in register, but are rotated one with respect to the other.

The columns are similar to the four-stranded helix formed by poly(G) whose structure was studied by X-ray diffraction [5]. At high concentration they self-correlate to give cholesteric phases; at higher concentrations, more ordered phases are obtained. In the centre of the G-quartets, there is space to accommodate an ion of appropriate size, which interacts, via coordination, with the eight oxygen atoms of the eight surrounding guanines; in fact, the self-assembly process and the liquid crystal formation are deeply influenced by the nature of the ions present [15–17].

We report an investigation of the self-assembly process of the ammonium salts of four guanylic acids: guanosine 3′-phosphate (3′-GMP), guanosine 5′-phosphate (5′-GMP), guanosine 2′-phosphate (2′-GMP) and guanosine 3′:5′-cyclic phosphate (*c*GMP). These compounds attracted our attention as, although guanylic acid aggregates and gels have been extensively studied, up to now no evidence of the lyotropic mesomorphism of riboguanosine derivatives has been reported. We were curious to test whether the presence of the 2′-hydroxyl group could affect the self-assembly process exhibited by the analogous 2′-deoxy derivatives 3′-dGMP, 5′-dGMP and d*c*GMP [10, 11, 17–19].



2. Results and discussion

2.1. *Optical microscopy observations*

All the investigated compounds, when dissolved in water, exhibit lyotropic mesomorphism. The phase sequences exhibited at room temperature by the ammonium salts of the compounds investigated, as deduced from the typical textures shown by the different phases in polarized light, are the following:

3′-GMP:I—2·8%—Ch—10–15%—H
5′-GMP:I—1·8%—Ch—12–18%—H
2′-GMP:I—20%—I + Ch
*c*GMP: I—13–19%—Ch—>50%—H

where I, Ch and H represent the isotropic, cholesteric and hexagonal phase, respectively. In no case could the

* Author for correspondence.

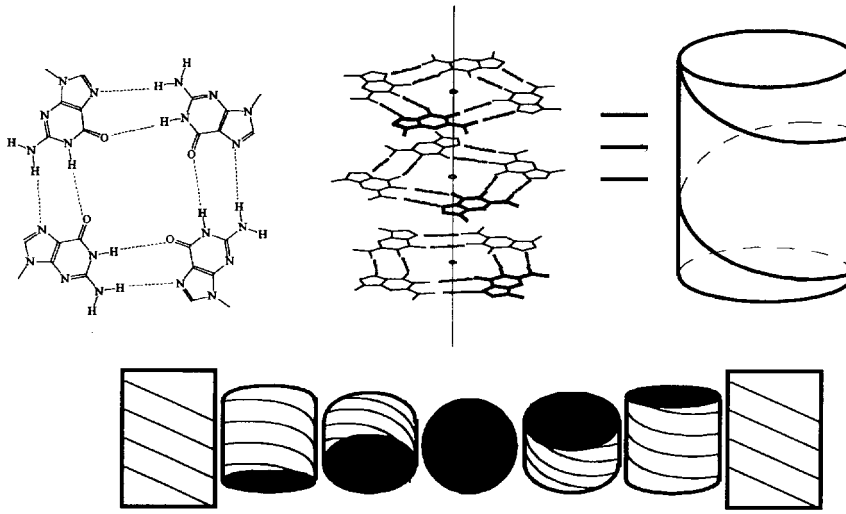


Figure 1. The assembly process of guanine derivatives and the formation of the cholesteric phase.

fingerprint or the planar texture of the cholesteric phase be obtained for thick samples, even after the samples had been kept in a 0·5 T magnetic field for a long time. The phase sequence of 5′-GMP has been confirmed by X-ray diffraction [20] and the structural features of its hexagonal phase fully agree with the model described in the introduction.

2.2. *Circular dichroism experiments*

Circular dichroism spectroscopy is very sensitive to stereochemical variations; it is ideal for following the aggregation process of GMPs from the isolated molecules to the supramolecular aggregates and finally to the cholesteric mesophases. Therefore CD spectra of aqueous solutions of ammonium salts of GMPs at different concentrations and temperatures and in the presence or absence of KCl were recorded; some selected spectra are reported in figure 2.

The solution behaviour of the compounds investigated is similar, and three characteristic spectra can be identified:

(i) The spectra of the free or isolated monomeric species (short-dashed lines in figure 2) can be observed in dilute solutions in pure water; the same spectra are obtained at higher temperature, in more concentrated solutions or in the presence of salts. For all the compounds, they show weakly dichroic signals at *c.* 250 nm and at *c.* 210 nm.

(ii) The spectra of the assembled species (solid lines in figure 2) are drastically different and are characterized by a relatively intense non-conservative, negative, exciton-like couplet [21] centred around 240–250 nm. Although the CD spectra of the assembled species obtained from the different compounds are not identical in shape, all are characterized by this typical feature. The amplitude of the couplet depends on the concentration of the guanine derivatives and on the salts added. In the case of 2′-GMP, this spectrum can be obtained only in the presence of 1M KCl and is superimposed on a signal due to scattering effects consequent upon incipient crystallization.

(iii) At much higher concentration (or lower temperature), an intense negative band (long-dashed lines in figure 2) may be observed: these spectra, whose shapes are similar to those of the absorption spectra, are associated with the formation of the cholesteric phase. In the case of 2′-GMP, it was not possible to obtain this spectrum, probably due to the fact that at the highest concentration accessible to spectroscopic determination, the system is biphasic, with a small amount of cholesteric phase (even at low temperature and in the presence of salt).

2.3. *The columnar helicity*

Several previous results are useful in interpreting the CD spectra of the aggregates. First, the CD spectra of the four-stranded helix of poly(G) and poly(dG) are known, and also the structure of the helix, similar to our columnar aggregates, is known in detail from fibre X-ray work on poly(G) [5]. Second, the spectroscopic properties of guanine have been extensively studied. Consequently, it is possible to calculate the CD of poly(G) and of other similar molecules using a relatively simple exciton treatment [22]. From this approach, the chirality of the columnar aggregates can be deduced. In the low energy part of the spectrum, the guanine chromophore displays two well-characterized electronic transitions corresponding to an absorption maximum at *c.* 250 nm and to a shoulder at *c.* 280 nm. In the case of poly(G), the transition at *c.* 250 nm gives rise to a non-symmetric positive exciton couplet; it follows that, whenever spectra similar to this are obtained, they can be related to a right-handed four-stranded structure.

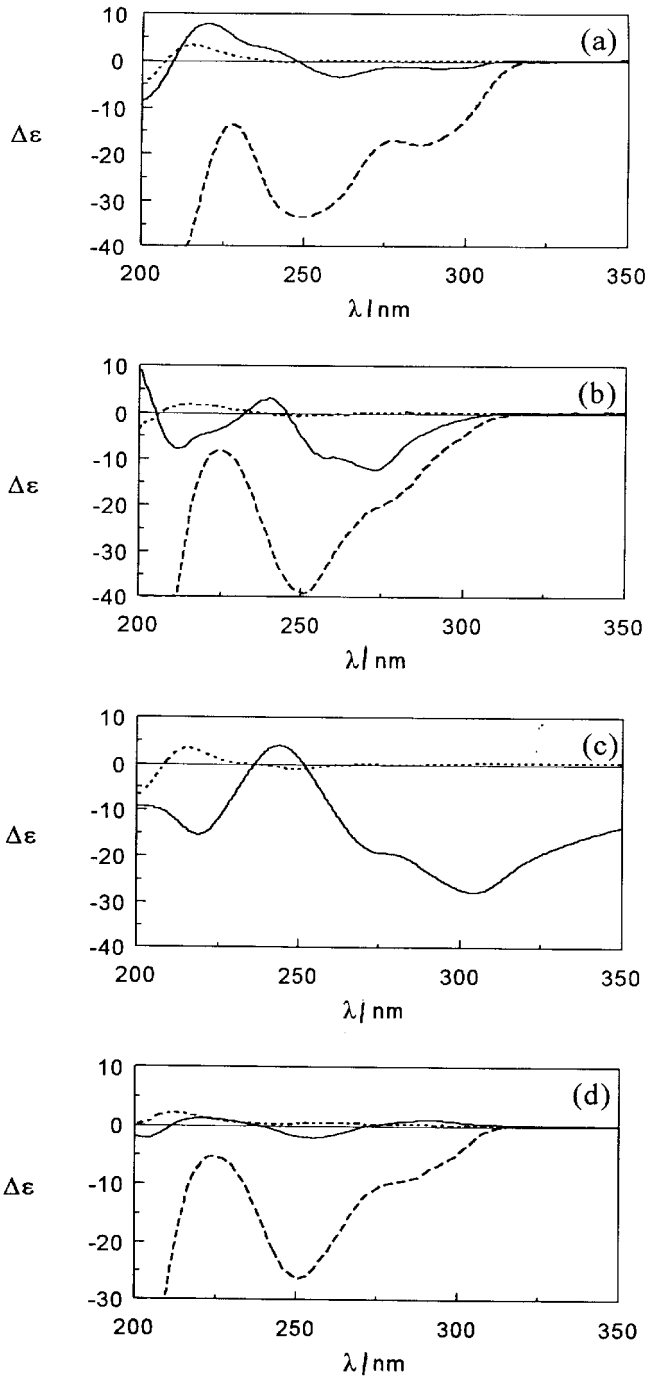


Figure 2. A few selected Circular Dichroism spectra. (*a*) 3′-GMP: 3·2% in water at 20°C (long-dashed line), 0·4% in 0·5M KCl at 20°C (solid line) and 40°C (short-dashed line); (*b*) 5′-GMP: 2% in water at 20°C (long-dashed line), 0·4% in 0·1M KCl at 20°C (solid line) and 80°C (short-dashed line); (*c*) 2′-GMP: 0·4% in 1M KCl at 1°C (solid line) and 20°C (short-dashed line); (*d*) *c*GMP: 2% in 0·2M KCl at 5°C (long-dashed line), 20°C (solid line) and 40°C (short-dashed line).

This correlation has both empirical and theoretical validity.

For all derivatives, the aggregates have CD spectra that are quasi-specular to that of poly(G), and left-handed columnar helicity can be inferred. The same helicity has been found for the corresponding deoxy derivatives [11, 17].

2.4. *The cholesteric handedness*

An 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 [23]; this also allows determination of the cholesteric handedness. A measurement of CD on a planarly aligned sample gives a signal $(A_l - A_r)_j$, which is correlated to the cholesteric characteristics by equation (1):

$$(A_l - A_r)_j = \tfrac{1}{2}\, p\, \nu_j^3 \Delta n (A_\parallel - A_\perp)_j / (\nu_j^2 - \nu_0^2) \qquad (1)$$

where ν_j is the frequency at which the measurement is carried out, ν_0 is the wavenumber of the selective reflection of the cholesteric. Δn and $(A_\parallel - A_\perp)_j$ are the linear birefringence and the linear dichroism, respectively, of the individual columns which compose the cholesteric phase; p is the helical pitch, positive or negative for a right-handed or left-handed cholesteric, respectively. The frequency ν_0 in all cases is much smaller than ν_j; the linear birefringence and dichroism are both negative as guanine has electronic transitions polarized in the molecular plane (i.e. perpendicularly to the cylinder axis). A positive CD signal in the absorption region therefore indicates a right-handed cholesteric, and a negative CD indicates a left-handed cholesteric.

For compounds 3′-GMP, 5′-GMP and *c*GMP, the cholesteric phases are left-handed. The handedness of 2′-GMP was not determined as it was not possible to obtain a cholesteric phase in the thin cell used for CD measurements.

3. Conclusions

The ability of 5′-GMP to self-assemble has been known since the early twentieth century [2]. In the 60s and 70s, many reports appeared on gel formation from the isomeric guanylic acids [3]. Later, attention was focused on the self-assembled structures present in isotropic solution [7]. The present paper reports the first description of the appearance of lyotropic mesophases from ribonucleotides.

The finding that 3′-, 5′- and 2′-GMP, as well as the 3′:5′-cyclic derivative *c*GMP, form, with increasing concentration, self-assembled species and finally liquid crystal phases, indicates that the presence of a hydroxyl group (also phosphorylated) in the 2′ position does not inhibit the formation of the mesophase already reported for 3′-dGMP, 5′-dGMP and d*c*GMP. This common

behaviour is reflected also in the same handedness of adjacent guanine tetramers and the same cholesteric sense.

It is worth mentioning that, for compound 5′-GMP, the formation of gels was reported [6] for concentrations (0·03 M in 0·1 M KCl) at which, in the present research, cholesteric phases have been described. This is not surprising since in the 70s, the study of columnar liquid crystalline cholesterics had not yet been initiated.

4. Experimental

4.1. *Materials*

All GMP compounds were commercial products from Sigma.

4.2. *CD experiments*

CD spectra were recorded with a JASCO J710 spectropolarimeter equipped with a Neslab circulatory thermostat (thermal stability 1°C) and using 0·001–0·01 cm cells with a thermostatting jacket. Solutions were prepared by dissolving the compounds (0·4–4% w/w) in an aqueous solution of KCl (0–1M). 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 a longer time). Concentrations of the GMPs were determined spectrophotometrically at 80°C (ε 13700 for 5′-GMP, 3′-GMP and *c*GMP, ε 13300 for 2′-GMP in relation to λ_{max} around 251–253 nm) 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*

Isotropic→cholesteric→hexagonal transitions were detected by the texture exhibited by the relevant solutions when inserted between a microscope slide and coverslip and observed with a Zeiss polarizing microscope.

We thank Dr. Anna Garbesi (I.Co.C.E.A.–C.N.R., Bologna) for helpful discussions and Dr. Paolo Mariani (University of Ancona) for the preliminary results of X-ray diffraction. Financial support from C.N.R. and M.U.R.S.T. (Italy) is gratefully acknowledged.

References

[1] For a commentary, see Williamson, J. R., 1993, *Proc. Natl. Acad. Sci. USA*, **90,** 3124.
[2] Bang, I., 1910, *Biochem. Z.*, **26,** 293.
[3] Guschlbauer, W., Chantot, J.-F., and Thiele, D., 1990, *J. biomol. Struc. Dyn.*, **8,** 491.
[4] Laughlan, G., Murchie, A. I. H., Norman, D. G., Moore, M. H., Moody, P. C. E., Lilley, D. M. J., and Luisi, B., 1994, *Science*, **265,** 520.
[5] Arnott, S., Chandrasekaran, R., and Martilla, C. M., 1974, *Biochem. J.*, **141,** 537;
Zimmermann, S. B., Cohen, G. H., and Davis, D. R., 1975, *J. mol. Biol.*, **92,** 181.
[6] Chantot, J.-F., Haertle, T., and Guschlbauer, W., 1974, *Biochemie*, **56,** 501, and references therein.
[7] See, e.g. Fisk, C. L., Becker, E. D., Miles, H. T., and Pinnavaia, T. J., 1982, *J. Am. chem. Soc.*, **104,** 3307.
[8] Attwood, T. K., Lydon, J. E., and Jones, F., 1986, *Liq. Cryst.*, **1,** 499.
[9] Mariani, P., Mazabard, C., Garbesi, A., and Spada, G. P., 1989, *J. Am. chem. Soc.*, **111,** 6369.
[10] Bonazzi, S., Capobianco, M., De Morais, M. M., Garbesi, A., Gottarelli, G., Mariani, P., Ponzi Bossi, M. G., Spada, G. P., and Tondelli, L., 1991, *J. Am. chem. Soc.*, **113,** 5809.
[11] Spada, G. P., Bonazzi, S., Garbesi, A., Zanella, S., Ciuchi, F., and Mariani, P., 1997, *Liq. Cryst.*, **22,** 341.
[12] Gottarelli, G., Spada, G. P., and Garbesi, A., 1996, *Comprehensive Supramolecular Chemistry*, Vol. 9, edited by J.-M. Lehn *et al.* Oxford: Pergamon Press, Chap. 13.
[13] Carsughi, F., Ceretti, M., and Mariani, P., 1992, *Eur. Bioph. J.*, **21,** 155.
[14] Gottarelli, G., and Spada, G. P., 1994, *Circular Dichroism: Interpretation and Applications*, edited by K. Nakanishi, N. Berova and R. W. Woody (New York: VCH), Chap. 5.
[15] Pinnavaia, T. J., Marshall, C. L., Mettler, C. M., Fisk, C. L., Miles, H. T., and Becker, E. D., 1978, *J. Am. chem. Soc.*, **100,** 3625; Detellier, C., and Laslo, P., 1980, *ibid.*, **102,** 1135.
[16] Bonazzi, S., Gottarelli, G., Spada, G. P., Mariani, P., Romanzetti, S., Garbesi, A., and La Monaca, A., 1995, *Gazz. Chim. (It.)*, **125,** 483.
[17] Gottarelli, G., Proni, G., and Spada, G. P., 1996, *Enantiomer*, **1,** 201.
[18] Mariani, P., De Morais, M. M., Gottarelli, G., Spada, G. P., Delacroix, H., and Tondelli, L., 1993, *Liq. Cryst.*, **15,** 757.
[19] Franz, H., Ciuchi, F., Di Nicola, G., De Morais, M. M., and Mariani, P., 1994, *Phys. Rev. E.*, **50,** 395.
[20] Mariani, P., (to be published).
[21] Mason, S. F., 1982, *Molecular Optical Activity and the Chiral Discrimination*, (Cambridge: Cambridge University Press).
[22] Gottarelli, G., Palmieri, P., and Spada, G. P., 1990, *Gazz. Chim. (It.)*, **120,** 101.
[23] Dudley, R. J., Mason, S. F., and Peacock, R. D., 1975, *J. chem. Soc., Faraday Trans. 2*, 997.