OPTICAL ACTIVITY OF 5'-GUANYLIC ACID GEL*

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We have shown recently that the ORD[1] of deoxyribo- and ribo-mononucleotides displays a single Cotton effect between 240 and 300 mμ, positive for the pyrimidine and negative for the purine ones (Yang and Samejima, 1963; Yang, Sarkar, and Samejima, unpublished results). In contrast, the nucleic acids (Samejima and Yang, 1964, 1965) and the synthetic polynucleotides (with one exception) and their complexes (Sarkar and Yang, 1965a, b) all show two peaks and one trough above 220 mμ, are dextrorotatory in the visible region, and their magnitudes are very large as compared with those of their constituent mononucleotides. The striking exception is poly I which has two troughs and one peak between 240 and 300 mμ and is levorotatory in the visible region. Since inosine is an analogue of guanosine, we studied the rotatory properties of GMP gel, because poly G is very difficult to prepare. X-ray diffraction studies show GMP to form a left-handed helical structure

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[1] Abbreviations: ORD, optical rotatory dispersion; GMP, 5'-guanylic acid; poly G, polyguanylic acid; poly I, polyinosinic acid.

(Gellert et al., 1962). Such base stacking and interaction in a helical structure is expected to give rise to multiple Cotton effects (Tinoco, 1964). Our results show that GMP gel does display an ORD profile similar to that of poly I, even though the monomers are not linked covalently.

Optical Rotatory Dispersion. - A solution of 10 mg/ml or more of GMP in 0.01 M acetate buffer plus 0.2 M NaCl (pH 5) forms a clear gel when cooled from room temperature to 1-2° C. This is accompanied by drastic changes in the optical properties of GMP (Figs. 1 and 2; Table I).

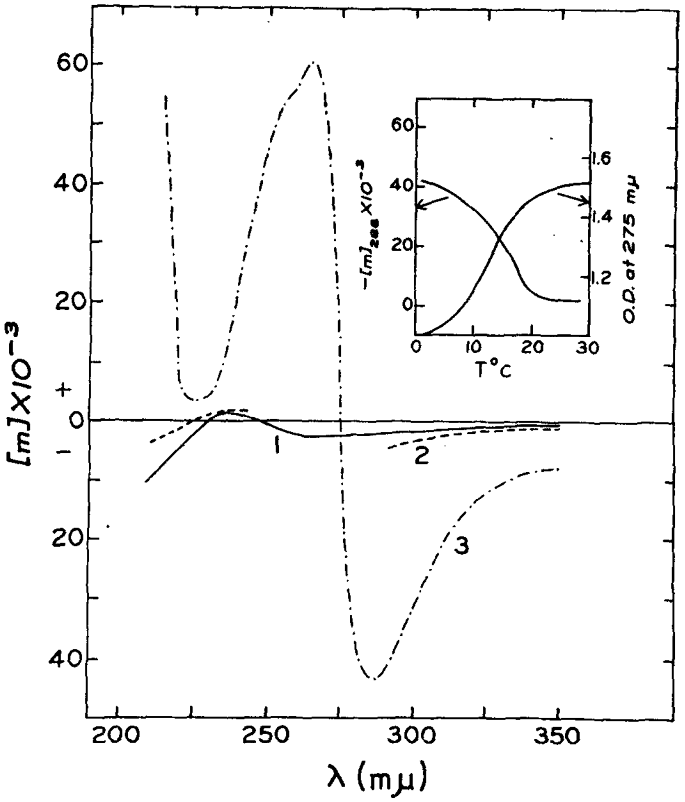

Fig. 1. Ultraviolet rotatory dispersion of 5'-guanylic acid. Curve (1) 5 mg/ml, 27° C; (3) 10 mg/ml, 2° C; and (2) 10 mg/ml, warmed from 2 to 20° C, experimental points between 240 and 290 mμ being omitted because of high absorbance (O. D. >2). Insert: the melting curve of the gel.

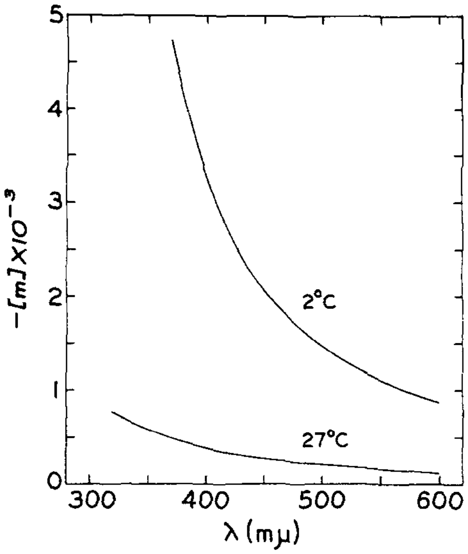

Fig. 2. Visible rotatory dispersion of 5'-guanylic acid (10 mg/ml).

Table I. Rotatory parameters of 5'-guanylic acid solution and gel (10 mg/ml)$^+$

Temperature, °C.	λ_t, mμ	$[m]_t$	λ_p, mμ	$[m]_p$	λ_t, mμ	$[m]_t$	λ_c, mμ	k
		$\times 10^{-3}$		$\times 10^{-3}$		$\times 10^{-3}$		$\times 10^{-6}$
27	260	-2	235	+1	-	-	221	-42
2	288	-43.5	265	+61.0	225	+3.5	295	-236

$^+$[m] = mean residue rotation; p and t refer to the peak and trough.

Three features immediately emerge: First, the ultraviolet rotatory dispersion of GMP at 2° C shows a tremendous increase in the magnitude of rotation at the peaks and troughs and displays multiple Cotton effects, suggesting strong base stackings in the gel. In contrast, only a single Cotton effect was observed above 240 mμ at 27° C. Furthermore, the ORD profile of the gel resembles that of helical poly I with two troughs and one peak (Sarkar and Yang, 1965b), but the Cotton effects of the gel are much larger (for poly I in 1 M NaCl at pH 7, the [m]'s at the 288 mμ trough, 265 mμ peak, and 245 mμ trough were -9.7, +13.5, and -14.5 x 10^3, respectively).

Second, both the peaks and troughs in Fig. 1 are very sensitive to temperature, and the formation of GMP gel is reversible. The variation of the 288 mμ trough with temperature (see the insert, Fig. 1) parallels the change in absorbance at 275 mμ as observed by Gellert _et al._ (1962); the melting temperature is about 15° C.

Third, the drastic changes in the Cotton effects (Fig. 1) are reflected in the visible rotatory dispersion (Fig. 2). The greatly increased levorotation at 2° C again is in accord with the attainment of ordered structure in the gel. The data at both temperatures obey a one-term Drude equation: $[m] = k/(\lambda^2 - \lambda_c^2)$ (Table I).

Ultraviolet Spectra (Fig. 3). - At room temperature, GMP

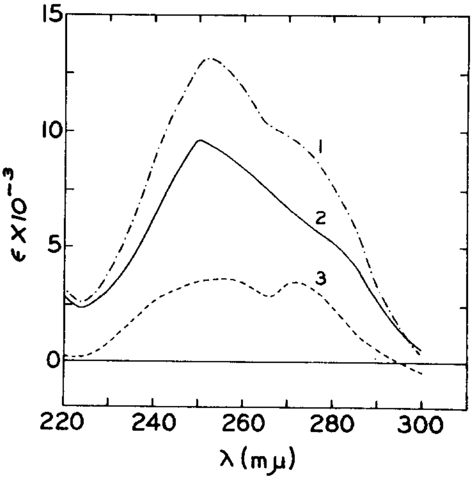

Fig. 3. Absorption spectra of 5'-guanylic acid. Curve (1) 27° C; (2) 2° C; and (3) difference between (1) and (2).

showed an absorption maximum at 253 mμ with a shoulder near 265 mμ. About 40 % hypochromism was observed after the gel formation, and the absorption maximum underwent a blue shift to 250 mμ and the shoulder a red shift to 280 mμ. Consequently, the difference spectra show two peaks at 272 and 256-257 mμ, in good agreement with the finding of Gellert et al. (1962).

The X-ray diffraction study of GMP fiber prepared from its gel is consistent with a left-handed continuously hydrogen-bonded helical model with 15 GMP per 4 turns (Gellert et al., 1962; D. R. Davies, private communication). However, results of a similar study on poly I fiber (Rich, 1958) are inconsistent with a left-handed helical structure, although the ORD profiles of both GMP gel and poly I are very similar to each other. As Tinoco (1964) pointed out, the sign of the Cotton effects is determined by the potential of interaction of groups involved in transition and the number of such groups per turn of the helix. Our present knowledge of the nucleotides and polynucleotides does not permit us to distinguish the handedness of their helical structures. In this respect we note the recent report on isoguanosine, which also forms aggregates in high concentration and at low temperature (Ravindranathan and Miles, 1965): this nucleoside has a dextrorotation in the visible region, which is markedly increased with the formation of gel. Perhaps the Cotton effect profile of the isoguanosine gel will be opposite to those reported herein.

Experimental. - 5'-Guanylic acid was purchased from the California Corp. for Biochemical Research. Its concentration was determined spectrophotometrically using a molar absorptivity of 13.5 x 10^3 at 252.5 mμ at 25° C (Voet et al., 1963).

Optical rotatory dispersion was measured with a Cary 60 spectropolarimeter using water-jacketed cells having light paths of 0.1 mm and 1 cm. Details of the procedures have been described (Sarkar and Yang, 1965a). At 27° C, the absorbance of 1 % GMP was too strong to be measured between 240 and 290 mμ; accordingly, the concentration in this case was reduced to one half. At 2° C, however, considerable hypochromism due to the gel formation made it possible to measure the 1 % solution. To reduce the noise level of the instrument, we set the slit width of the monochromator at 2 mm throughout the ultraviolet region; thus, the band widths at, say, 350, 275, and 210 mμ were 10, 4.4, and 1.4 mμ, respectively. All the rotations were expressed in terms of molar rotation, [m].

Absorption spectra were measured in a Cary 14 spectrophotometer using a 0.1 mm cell and expressed in terms of molar absorptivity, ϵ. At 27° C, the 1 % GMP solution was diluted to one-fifth to lower the absorbance well below 2.

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