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On Self-Interacting Oligoribonucleotides. I. Absorption and Optical Rotatory Dispersion of 2′–5′- and 3′–5′-Oligoguanylic Acids*

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ABSTRACT: A series of 2′–5′-oligoguanylic acids are prepared by reacting G(cyclic)p with takadiastase T_1 ribonuclease and separating the products chromatographically. The 3′–5′-oligoguanylic acids are obtained by separating the products of alkaline degradation of 3′–5′-poly(G). The optical rotatory dispersion and hypochromism of both 2′–5′- and 3′–5′-oligoguanylic acids are studied at two different pH. The optical rotatory dispersion spectrum of 2′–5′-GpG is significantly different from that of 3′–5′-GpG. The magnitude of rotation of the long-wavelength peak of 2′–5′-GpG is larger than that of 3′–5′-GpG. This finding contradicts the explanation that the extra stability and more intense circular dichroism band of other 3′–5′-dinucleoside monophosphates is due to H-bond formation between 2′-OH and either the base or the phosphate oxygen. The end phosphate group has a marked effect on the spectrum of GpG between 230 and 250 mμ. In addition the optical rotatory dispersion spectra of 2′–5′ exhibit strong pH, temperature, and solvent dependence between 230 and 250 mμ. ΔH and ΔS for order $\rightleftharpoons$ disorder transition is estimated to be 9.7 kcal/mole and 35.2 eu, respectively. The optical rotatory dispersion spectra of guanine-rich oligoribonucleotides, GpGpC, GpGpU, GpGpGpC, and GpGpGpU are compared to the calculated optical rotatory dispersion from the semiempirical expression of Cantor and Tinoco, using measured optical rotatory dispersion of dimers. Contrary to previous studies, agreement is found not at all satisfactory.

However, optical rotatory dispersion of 3′–5′-GpGpGpC and GpGpGpU can be estimated from the semiempirical expression, if a next-nearest interaction parameter is introduced empirically. Such interaction parameter can be calculated from the measured properties of trinucleotide sequences like GpGpG, GpGpC, and GpGpU, assuming that only the nearest-neighbor interaction is important. The optical rotatory dispersion of single-stranded poly(G) is also predicted. The importance of syn–anti equilibrium and next-nearest-neighbor interaction in oligoguanylic acids is suggested as a probable explanation.

The stacking interaction is shown to be major source of conformational stability in helical polynucleotides (Van Holde *et al.*, 1965; Brahms *et al.*, 1966). Tinoco and his coworkers have shown that the optical properties such as absorption and optical rotatory dispersion of polynucleotides, particularly in the single-stranded conformation, is mainly influenced by its nearest-neighbor residue. Thus optical rotatory dispersion and absorption spectra of any single-stranded polynucleotide of known sequence can be empirically calculated from the corresponding measured properties of 16 dinucleotides (Cantor and Tinoco, 1965). Similarly the optical properties of double helices of any defined sequence can be expressed in terms of measured properties of the different dimer pairs (Cantor *et al.*, 1966). Jaskunas *et al.* (1968) have attempted to obtain the optical rotatory dispersion of different dimer pairs from the study of complexes formed between complementary trinucleotides in 10^{-2} M solutions in 0.1 M phosphate buffer containing 0.5 M NaCl (pH 7.0). Under those conditions, among the various trinucleotides studied, only GpGpC interacts with itself to form large aggregates as well as with its complementary GpCpC to form a series of higher order complexes with an average stoichiometry of 2:1. These approaches were then extended to the study of complexes between complementary tetranucleotides as one would expect that decrease in the extent of aggregation with increasing chain length might favor the formation of well-defined complexes containing complementary base pairs. Contrary to our expectation, the optical rotatory dispersion of GpGpGpU and GpCpCpA alone and of the mixture of the two indicated no complex formation to have occurred under those conditions. It has been shown later that GpGpGpU associates themselves strongly even in 10^{-5} M solution. The rates of formation and of dissociation of such self-associated complexes of GpGpGpU are extremely slow (compare rates of A–U and G–C base-pair formation, Podder, 1971) and thereby limits the formation of complementary base pairs. When this work was undertaken it was not known whether such slow rates are characteristics of G–G base pairs or intercalated complexes. The presence of uracil residue at the end might cause unstacking thereby favoring intercalation (Chan and Nelson, 1969). To comment further, knowledge of physicochemical properties

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of oligo(G) and their aggregates is essential. Once this is known, the G–C helix of relatively short chain length can be investigated in detail in order to determine the thermodynamic and kinetic constants of a cooperative helix–coil transition. Since RNA is rich in G–C content, these constants are to be known precisely for understanding conformation and stability of RNA.

The homopolymer pairs such as poly(C):poly(G) and poly(C):oligo(G) have also been investigated earlier (Pochon and Michelson, 1965; Lipsett, 1964b), but they were not carried out in great detail and therefore the evaluation of the thermodynamic parameter was not made possible. A reinvestigation of these systems is felt necessary. For this purpose, an ascending series of oligo(G) was synthesized from G-cyclic-p by the action of ribonuclease T_1 according to the procedure in the literature (Lipsett, 1964a). Contrary to our expectation synthesized oligo(G) contained 2′–5′-phosphodiester which was found to be completely resistant to the action of ribonuclease T_1. The details of such studies were reported elsewhere (Podder and Tinoco, 1969; Podder, 1970). The present investigation which deals with a comparative study of structure and stability of 3′–5′- and 2′–5′-oligoguanylic acids, was undertaken to understand the role of 2′-OH in the conformational stability of RNA. In addition the measu ed optical rotatory dispersion spectra of guanine-rich oligonucleotides is compared to the spectra calculated on the basis of nearest-neighbor approximation (Cantor and Tinoco, 1965). It is shown that nearest-neighbor approximation is too crude to predict the optical properties of guanine-rich oligonucleotides in their single-stranded conformation.

Materials and Methods

Materials. The synthesis of a series of 2′–5′-oligoguanylic acids was done as described previously (Podder, 1970). The reaction mixture contained 0.15 M G-cyclic-p and 800 units/ml of ribonuclease T_1 (Calbiochem lot 71020) in pH 7, Tris buffer. The mixture was kept at room temperature for 18–24 hr. It was then heated in boiling water for 5 min to inactivate the enzyme and diluted to 1:1 by volume with 7 M urea. The mixture was brought to pH about 1 for 3–4 hr to open any cyclic phosphates. Finally oligomers were separated by chain length on a DEAE-Sephadex–urea column. Measurable amounts of oligomers of chain up to 5 were obtained. The chain length in each peak was determined from the ratio of nucleotide to nucleoside found after dephosphorylation and alkaline hydrolysis of the dinucleotide fraction. Dimers and trimers were desalted on a DEAE-Sephadex–$(NH_4)_2CO_3$ column and lyophilized. Tetramer and higher oligomers were desalted by dialysis. The chain length of each peak was further confirmed by determining their molecular weights under conditions that disfavored aggregation. The oligo(G) thus synthesized were completely resistant to the ribonuclease T_1 hydrolysis and contained only 2′–5′-phosphodiester linkages. These were further confirmed by specific chemical degradation of 2′–5′-GpG into 2′-GMP (Podder and Tinoco, 1969). 2′–5′-GpG was obtained by dephosphorylating the dinucleotide 2′–5′-GpGp with *E. coli* alkaline phosphatase at 45°. The dephosphorylated mixture was then separated on DEAE-Sephadex–$(NH_4)_2CO_3$ column as before by eluting with $(NH_4)_2CO_3$ and lyophilized.

The 3′–5′-GpG was obtained by dephosphorylation of GpGp which was isolated from the products of alkaline degradation of poly(G) (Miles, control 11-4-314). The 3′–5′-GpGpG was purchased from Miles. GpGpGpU and GpGpGpC were obtained from pancreatic digest of yeast RNA by known procedure (Rushizky and Sober, 1964; Jaskunas *et al.*, 1968). The products were characterized by determining their base composition and the ratio of nucleotide to nucleoside after alkaline hydrolysis.

Methods. MOLECULAR WEIGHT MEASUREMENTS. A Beckman ultracentrifuge Model E, equipped with modified temperature control (Smiriga and Hearst, 1969; Gray and Hearst, 1968) and a photoelectric scanner, was used for the measurement of sedimentation equilibrium. The absorbance of each sample 260 mμ was about 0.5–0.7. Though after 28-hr equilibration, scans appeared to reach a constant level, the final reading was taken after 36–40-hr equilibration. Several scans were made at the end of a run with 1–2-hr interval at slowest scan speed. The magnification factor, an absolute value of the radius, and the calibration of absorbance *vs.* pen deflection, were obtained as described in the manual for the scanner (E-TB-005). Several scans for each run were used to plot absorbance *vs.* $(\text{radius})^2$. The slopes of these plots always agreed within a few per cent (<4%) and these were averaged to calculate the experimental quantity ψ (see eq 1).

SPECTRAL MEASUREMENTS. The solutions of nucleotides (≃1 optical density unit) were prepared by dissolving the freeze-dried samples directly into appropriate buffers and were preequilibrated at 50–60° for 1–2 hr to avoid aggregation. The buffers used in the study were 10^{-2} M Tris-HCl, 0.05, and 0.1 M phosphate buffer (pH 7). Buffers of higher ionic strength were adjusted by addition of NaCl. The concentration of the preequilibrated solutions were then determined spectrophotometrically at room temperature using Cary 15, from its molar residue extinction coefficients at 260 mμ. Extinction coefficients of oligomers, longer than dinucleotide were either calculated from the extinction coefficients of monomer and dimer (Warshaw, 1966) using expression of nearest-neighbor approximation (Cantor and Tinoco, 1965) or determined experimentally (see later).

Optical rotatory dispersion measurements were made with a Cary 60 spectropolarimeter with circular dichroism attachment using thermostated cell holder. A small volume cylindrical cell (~1 ml) of 1-cm path length was used. In order to minimize evaporation above room temperature, the cell was lightly capped with a serum stopper which was previously boiled in 1.0 M KOH and rinsed with distilled water to leach out any ultraviolet-absorbing materials. The noise in the optical rotation signal was reduced by applying the smoothing procedure described by Savitzky and Golay (1964). To do so, the Cary 60–Cary 15 were connected to digital PDP 8-S computer, attached with a teletype. The computer recorded the arithmetic average of 150 points taken every 0.5 mμ. The data points were used in Savitzky and Golay 13-point smoothing procedure for a cubic function. A computer program was so written by Drs. M. Itzkovitz and B. Tomlinson (Tomlinson, 1968), that wavelength, raw data, and computed molar rotation from raw data were printed out while spectra were being recorded. Molar rotation per residue is defined as $\phi = 100\theta/Cl$, where θ is the measured rotation, l is the path length in centimeters, and C is the concentration in moles of mononucleotides (residue) per liter. The errors in the smoothed data are within ±5% of the average value.

THE DETERMINATION OF EXTINCTION COEFFICIENT. The extinction coefficient of 2′–5′- and 3′–5′-oligoguanylic acids were determined by hydrolyzing them to monomer with alkali and ribonuclease T_1, respectively, and measuring the change in absorbance for the process. The absorbance of preheated solutions of oligonucleotide in 0.05 M phosphate buffer

TABLE I: Molecular Weights by Sedimentation Equilibrium.[a]

Compound	Formula Wt	Concn (Mole/l.)	Measured Mol Wt (°C)	$\phi_p \times 10^{-4}$ (mμ)	$\phi_t \times 10^{-4}$ (mμ)	Condn for Mol Wt Measurements
3′–5′-$(Gp)_3U$	1279	5×10^{-5}	1200 (23)	0.42 (272)	−0.59 (250)	In 0.1 M phosphate–0.5 M NaCl (pH 7); solution is preheated for 2–3 hr at 50–60°.
	1279	5×10^{-5}	7344 (24)	6.4 (275)	−5.0 (250)	In 0.1 M phosphate–0.5 M NaCl (pH 7); freshly dilute solution from the stock solution (10^{-3} M), stored under frozen condition.
2′–5′-$(Gp)_4$	1379	6.4×10^{-5}	(a) 1258 (20)	0.28 (280)	−0.25 (250)	(a) In 0.05 M phosphate (pH 7). Solution was preheated at 50–60° for 1–3 hr.
			(b) 2000 (20)	0.76 (275)	−1.05 (250)	(b) Solution a in 0.18 M NaCl. Solution was frozen before molecular weight was measured.
			(c) 3400 (20)			(c) Solution a in 0.4 M NaCl. Solution was frozen before molecular weight was measured.

[a] The numbers in the parentheses (columns V and VI) refer to the position of the peak and trough of the optical rotatory dispersion spectra, measured at room temperature immediately after temperature equilibration.

was measured with a Cary 15 spectrophotometer coupled with PDP8/c teletype. About 1–2 ml of the solution is then transferred to a tube with a screw cap and small volume of 4 M NaOH (0.1–0.2 ml) was added to it such that final concentration of alkali was about 0.3–0.4 M. The tube was then tightly capped and was placed in 37° water bath for 18–20 hr for hydrolysis. At the end of incubation, the tubes were cooled in ice and spurred down to remove drops of solution adhering to lids. That no loss took place during incubation was checked by weighing the sample before and after. The caps were then carefully removed and an equivalent amount of 4 M $HClO_4$ in 0.05 M phosphate buffer was added to bring the pH of the solution around 7 (Richards, 1968). The absorbance of the hydrolyzed solution was measured against a buffer solution which was treated similarly. In case of 3′–5′-oligoguanylic acids absorbance of nucleotide in Tris buffer was measured before and after addition of ribonuclease T_1. All absorbance data were punched into tapes for the purpose of calculation of oscillator strength.

Results

Molecular Weight of Oligomers. The formation and dissociation of self-associated complexes of guanine-rich oligonucleotides occur slowly with the concomitant changes in the absorption and optical rotatory dispersion spectra. Since concentration, ionic strength, and temperature would strongly influence the extent of association, it is important to find the conditions that will favor only single-stranded conformations, so that the measured properties can be compared to the calculated values. In combination of optical rotatory dispersion measurements, the sedimentation equilibrium experiments were therefore performed with different oligomers under various sets of conditions. Molecular weight of the samples was calculated from eq 1 assuming that charge effects are negligible.

$$\psi = \frac{2RT}{w^2} \frac{\mathrm{d} \ln C_2}{\mathrm{d}r^2} = M_2(1 - \bar{v}\rho) \qquad (1)$$

where R is gas constant, T absolute temperature, r the distance from the center of rotation, ρ is the density of solution, w is the angular velocity, and $\bar{v}$ the partial specific volume (=0.53) (Tennent and Vilbrandt, 1943).

The molecular weight data are summarized in Table I, along with the magnitude of peak, ϕ_p, and trough, ϕ_t, of optical rotatory dispersion spectra. The data in Table I definitely suggest that such oligonucleotides form large aggregates even in dilute solution (10^{-4} M), particularly at low temperature. The physicochemical studies of the aggregates might be valuable as they might yield information about non-Watson–Crick base pairs. Therefore the optical rotatory dispersion of the aggregates and the rates of formation and dissociation of such aggregates have been subjected to the detailed investigations and will be reported elsewhere. These studies revealed that in dilute solutions the formation of self-associated complex takes place below room temperature and the rate of dissociation of aggregates into monomeric oligomer is slower than those of double helices containing Watson–Crick G–C and A–U base pairs by several orders of magnitude (Podder, 1971). For example, $t_{1/2}$ of the dissociation of GpGpGpU aggregates into its monomer is about 15–20 min at 50–60° in medium of 0.1 M phosphate buffer containing 0.5 M NaCl.

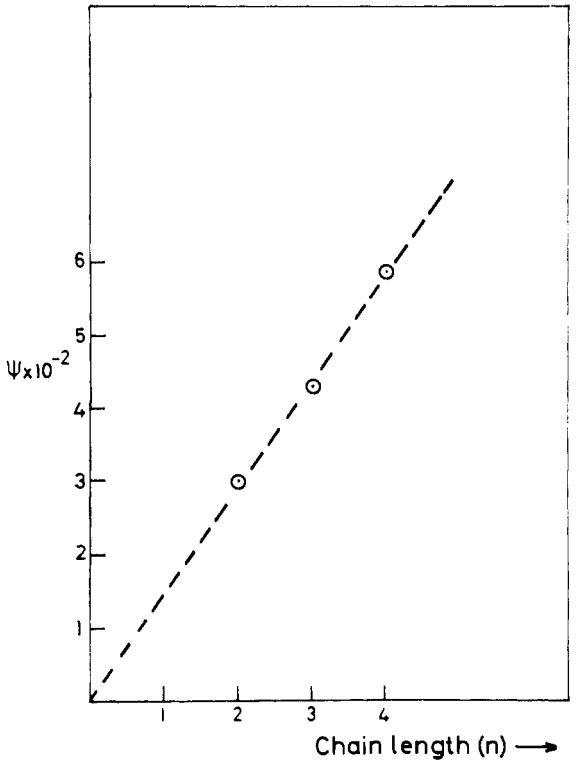

FIGURE 1: Ultracentrifugal molecular weights at neutral pH (0.05 M phosphate buffer) against chain length, n.

Figure 1 shows the plot of ψ of various oligoguanylic acids *vs.* assigned values of n. A straight line fits the data and thereby indicates that a correct assignment of chain length was made. The apparent molecular weight of GpG in 10^{-4}–10^{-5} M solution in 0.1 M phosphate buffer at 4 and 40° was found to be 553 and 564, respectively.

Extinction Coefficients and Hypochromicity. The extinction coefficients of oligonucleotides (per mole of residue) are calculated from eq 2 (Cantor and Tinoco, 1965), where $A(\mathrm{Gp})_n$ and

$$\epsilon_{(\mathrm{Gp})n}(\lambda) = \frac{A_n(\mathrm{Gp})(\lambda)}{A(\mathrm{Gp})(\lambda)}\epsilon_{\mathrm{Gp}}(\lambda) \qquad (2)$$

$A(\mathrm{Gp})$ are the absorbance of oligonucleotides before and after hydrolysis, respectively. ϵ_{Gp} is the extinction coefficient of GMP, taken from the literature (Warshaw, 1966). In Table II the measured ϵ_{260} values of various oligoguanylic acids are listed.

TABLE II: Extinction Coefficients at 260 mμ and Per Cent Hypochromism.

Compound	$\epsilon_{260} \times 10^{-4}$	% H
3′–5′-GpG	1.116	5
3′–5′-GpGpG	1.12	3
2′–5′-GpG	1.09	5
2′–5′-$(\mathrm{Gp})_2$	1.058	8
2′–5′-$(\mathrm{Gp})_3$	1.047	6
2′–5′-$(\mathrm{Gp})_4$	1.033	12

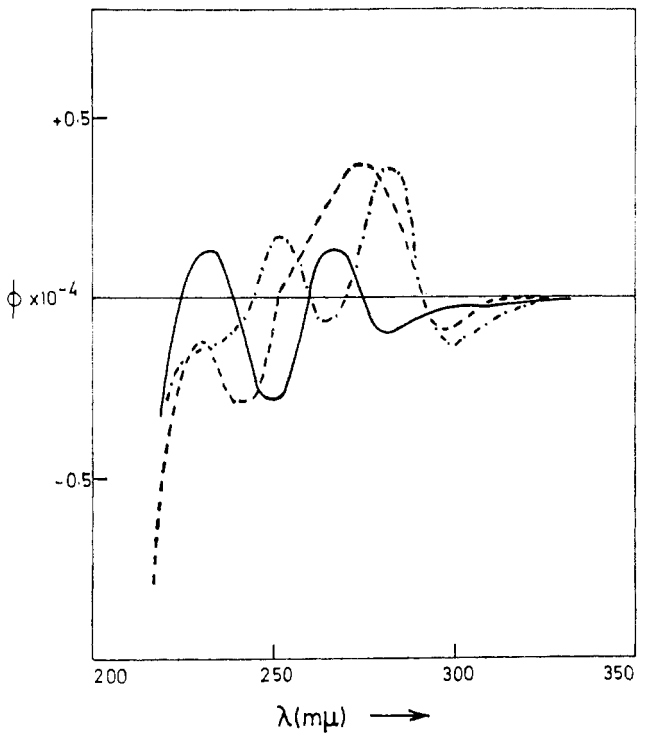

FIGURE 2: Optical rotatory dispersion of 2′–5′-GpG in 10^{-2} M Tris-HCl (pH 7), room temperature. Concentration 7 × 10^{-5} M (—·—). Optical rotatory dispersion of 2′–5′-GpGp in 0.05 M phosphate buffer (pH 7) at room temperature. Concentration 7 × 10^{-5} M (— —). Optical rotatory dispersion of 3′–5′-GpG in 10^{-2} M Tris-HCl (pH 7) at room temperature. Concentration 8.2 × 10^{-5} M (——).

The percentages of hypochromism given in Table II are computed from eq 3 as before (Warshaw and Tinoco, 1966).

$$H = \left(1 - \frac{f(\mathrm{Gp})_n}{f(\mathrm{Gp})}\right) \times 100 \qquad (3)$$

where $f(\mathrm{Gp})_n$ ($n > 2$) and $f(\mathrm{Gp})$ are oscillator strength of oligoguanylic acids and GMP, respectively. The oscillator strength is defined as

$$4.32 \times 10^{-9} \int_{\lambda_1}^{\lambda_2} \frac{(\epsilon\lambda)}{\lambda^2} d\lambda$$

where λ_2 (cm) is the long-wavelength limit where absorption has become zero and λ_1 (cm) the low-wavelength cutoff which is chosen arbitrarily around the absorption minimum (222 mμ). $f(\mathrm{Gp})_n$ and $f(\mathrm{Gp})$ were obtained from the integrated area of the absorption band before and after hydrolysis. The integration was done using Simson's rule on a PDP8/S computer. The absorption spectra of oligoguanylic acids are essentially same and are independent of phosphodiester linkages as well. The salient features are, however, hypochromicity in the vicinity of absorption band and hyperchromicity near the long-wavelength tail. Maximum hypochromicity is exhibited not around maximum but around shoulder (275 mμ). The percent hypochromicity is 4–5% and is lower than what one would expect on the basis that the guanine is likely to stack better. The results in Table II are probably accurate only within 1–2%, and therefore suggest that the dependence of hypochromicity on the chain length, if any, is not very large.

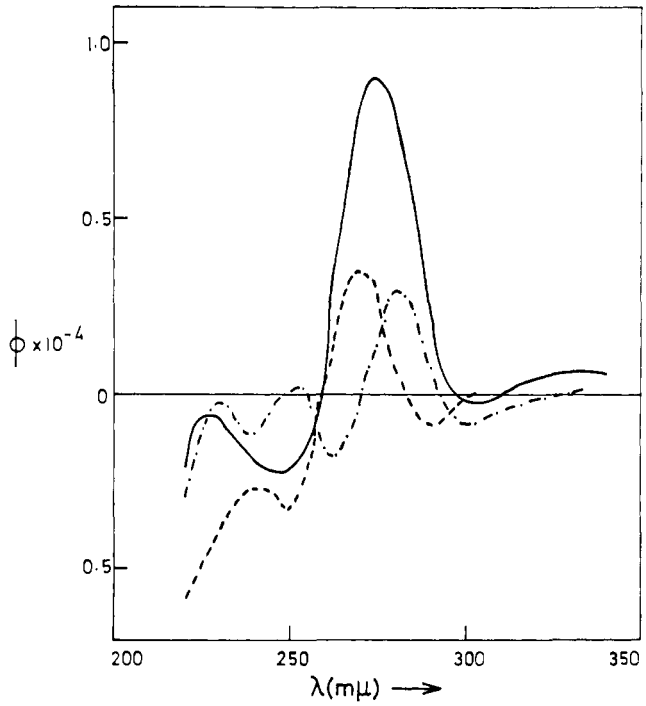

FIGURE 3: Optical rotatory dispersion of 2′–5′-GpG in 0.5 M HCl, at 4°. Concentration 6.7 × 10^{-5} M (——). Optical rotatory dispersion of 3′–5′-GpG in 0.5 M HCl, at 25°. Concentration 3.7 × 10^{-5} M (— —). Optical rotatory dispersion of 2′–5′-GpG in 25% methanol at 25°. Concentration 6.6 × 10^{-5} M (— · —).

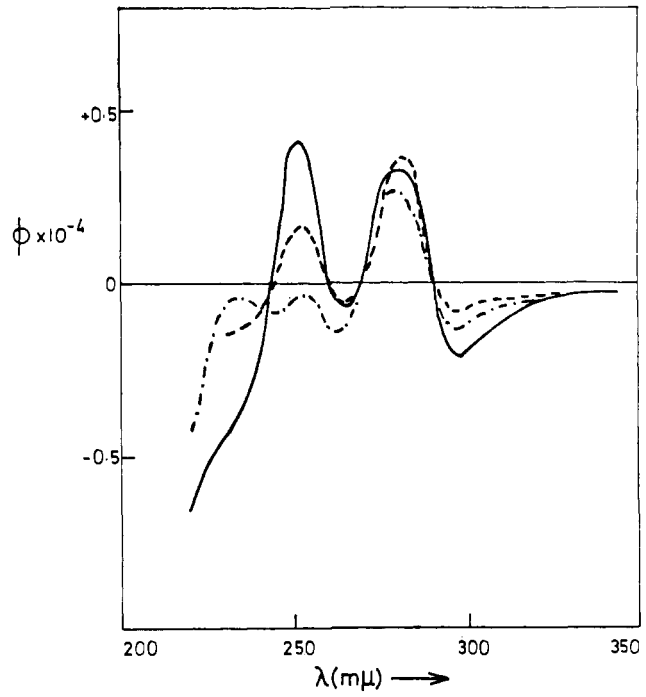

FIGURE 4: Optical rotatory dispersion of 2′–5′-GpG in 0.1 M phosphate buffer at three different temperatures. Concentration 7.2 × 10^{-5} M (——) 4°, (— —) 27°, and (— · —) 55°.

Optical Rotatory Dispersion of the Dinucleotides. In addition to absorption spectra, the optical rotatory dispersion spectra of 2′–5′-GpG, 2′–5′-GpGp, and 3′–5′-GpG in 10^{-2} M Tris-HCl buffer (pH 7) were measured at room temperature (Figure 2). The absorption spectra are quite similar to that of GMP (and therefore not shown). But the optical rotatory dispersion of each dimer is different qualitatively and quantitatively as well. The differences are not only reflected in general shape of optical rotatory dispersion curves but in position and sign of peak and trough. The absolute magnitude of rotation is comparatively small, although G is known to stack most.

Most striking, however, is the difference in optical rotatory dispersion of 2′–5′-GpGp and 2′–5′-GpG. The positive peak at 250 mμ of GpG is completely absent (Figure 2) in GpGp. Such an effect due to end phosphate group is observed for the first time. The effect is more pronounced as the temperature is lowered. That this is not due to self-association of GpG is checked from the measurement of optical rotatory dispersion in more diluted solution (using a 10-cm cell) and from determination of molecular weight from sedimentation equilibrium measurements. Molecular weight GpG at low temperatures was found to be within 10–15% of the formula weight. The optical rotatory dispersion of ApA resembles closely that of ApAp in shape and position of peak and trough but differs slightly in magnitude only (Davis, 1965). This is expected if repulsion causes only unstacking. In contrast, GpGp exist certainly in different conformation.

The optical rotatory dispersion of 3′–5′-GpG is different from that of 2′–5′ isomer. The position of peak and trough is shifted toward lower wavelength (Figure 2) and their intensity is comparatively small. In contrast, the intensity of circular dichroic bands of 3′–5′ isomers ApC, ApA, and CpC is larger than that of corresponding 2′–5′ isomers (Brahms *et al.*, 1967).

pH and Solvent Dependence. Figure 3 represents the optical rotatory dispersion of 2′–5′- and 3′–5′-GpG in 0.5 M HCl. Contrary to our expectation, they resemble optical rotatory dispersion of GpGp at neutral pH instead of that of GMP in acidic pH (Warshaw, 1966). This indicates that the base–base interaction is still present, although both bases are likely to be protonated, at such a low pH. Due to lack of pH titration data further comment is reserved. Most significant is, however, that the second peak of both GpG isomers disappears at low pH. Addition of methanol or rise in temperature results in a similar change (Figures 3 and 4). In general the second peak is found to be most sensitive to variation of solvent and temperature, whereas the long-wavelength peak and trough remain almost invariant under these conditions. Whether or not such dramatic changes can be correlated to change in conformation of bases relative to sugar in analogy with pH-induced conformational changes of guanosine monophosphate (Guschlbauer *et al.*, 1968) awaits further model theoretical calculations.

Thermal Denaturation. In order to calculate ΔH and ΔS for the stacking–unstacking process, optical rotatory dispersion of 2′–5′-GpG was measured as a function temperature (Figure 4). It is seen from Figure 4 that the magnitude of ϕ_{250} changes markedly while there is hardly any change in the long-wavelength peak and trough. The ultracentrifuge data indicate that these changes cannot be ascribed to the formation of self-associated complexes of GpG involving both base stacking and base pairing. Figure 5 shows ϕ_{250} as a function of temperature which is sigmoidal in shape and similar to those found for other dinucleoside monophosphate (Davis and Tinoco, 1968). At 55° molar rotation ϕ_{250} becomes negative and is equal to that of GMP in neutral pH and at room temperature (Yang *et al.*, 1966). However, the general shape of

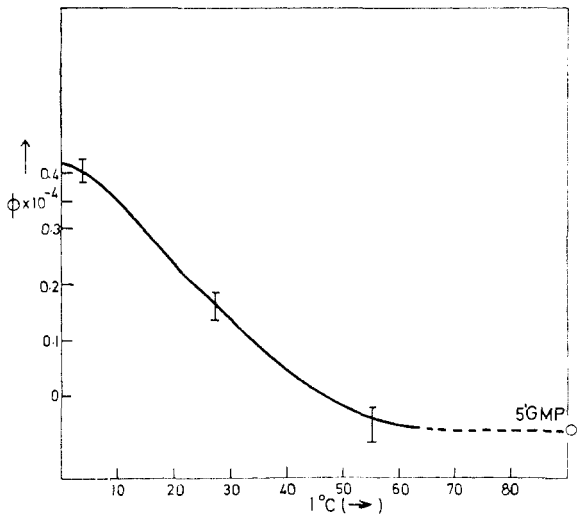

FIGURE 5: Molar rotation of 2′–5′-GpG at 250 mμ as a function of temperature.

the optical rotatory dispersion differs markedly from that of GMP. This fact imposes extra difficulty in estimating high-temperature limit of ϕ in addition to low-temperature value of molar rotation. Normally high-temperature limit is obtained from the rotation of components and the low-temperature limit is arbitrarily fixed in order to obtain a linear van't Hoff plot (Davis and Tinoco, 1968). For reasons above and because of the errors due to low signal-to-noise ratio, further measurements as a function of temperature were not carried out. However, an estimate of thermodynamic parameters

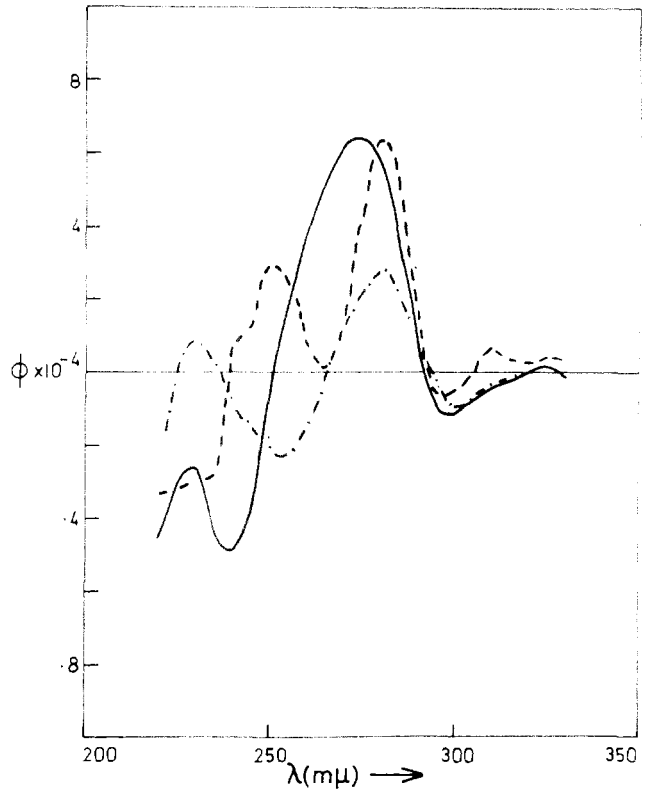

FIGURE 7: Optical rotatory dispersion of 2′–5′-$(Gp)_4$ in 10^{-2} M Tris buffer (pH 7) at room temperature. Concentration 9×10^{-5} M; measured (— · —) and calculated from eq 5, (——) with measured rotation of 2′–5′-GpGp and Gp; (— —) with those of 2′–5′-GpG and -Gp.

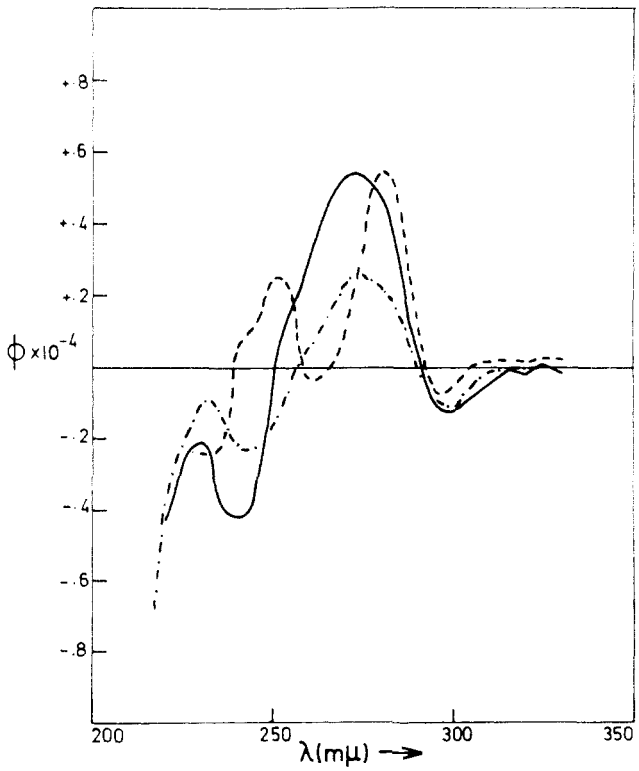

FIGURE 6: Optical rotatory dispersion of 2′–5′-$(Gp)_3$ in 10^{-2} M Tris-HCl (pH 7). Concentration 6.3×10^{-5} M measured (— · —) and calculated from eq 4, (——) with measured rotation of GpGp and Gp, (— —) with those of GpG and Gp.

can be made from the above data. Using ϕ of GMP ($=0.06 \times 10^4$) as an upper limit, a more or less linear van't Hoff plot is obtained with $\phi_{stack} = 0.9 \times 10^4$ from which ΔH and ΔS of stacking–unstacking process are evaluated as +9.7 kcal/mole and 35.2 cal/(deg mole), respectively. These values are not significantly different from those of 3′–5′-dinucleoside monophosphate. Thus it is not possible to draw any conclusion regarding relative stabilities of 2′–5′ isomer. If, on the other hand, the magnitude of rotation is taken as a measure of stacking interaction, the difference between the optical rotatory dispersion spectra of 2′–5′- and 3′–5′-GpG suggests itself that 2′–5′-GpG is more stacked at room temperature than 3′–5′-GpG (see Figure 2). In contrast, Brahms *et al.* (1967) concluded from the measurement of circular dichroism spectra of 2′–5′ derivatives ApC, ApA, and CpC and other 3′–5′-dinucleotides that 2′–5′ isomers are essentially in disordered conformation in the range of −20 to 8°, whereas corresponding 3′–5′ isomers are in the stacked conformation due to probable formation of H bond either with the base or phosphate oxygen. Recent studies with poly 2-*O*-methyladenylic acids do not confirm the above viewpoint. It is shown that the presence of unsubstituted 2′-OH group in poly(A) is not essential for the stabilization of single- or double-stranded conformation of poly(A) (Bobst *et al.*, 1969). The specificity, could, however, depend on intrinsic geometry of base and sugar which may vary due to specific solvation. Such a possibility cannot be completely ruled out unless one knows more about interaction of nucleotide with water.

Nearest-Neighbor Approximation. In sections to follow we shall report the measured optical rotatory dispersion of tri-

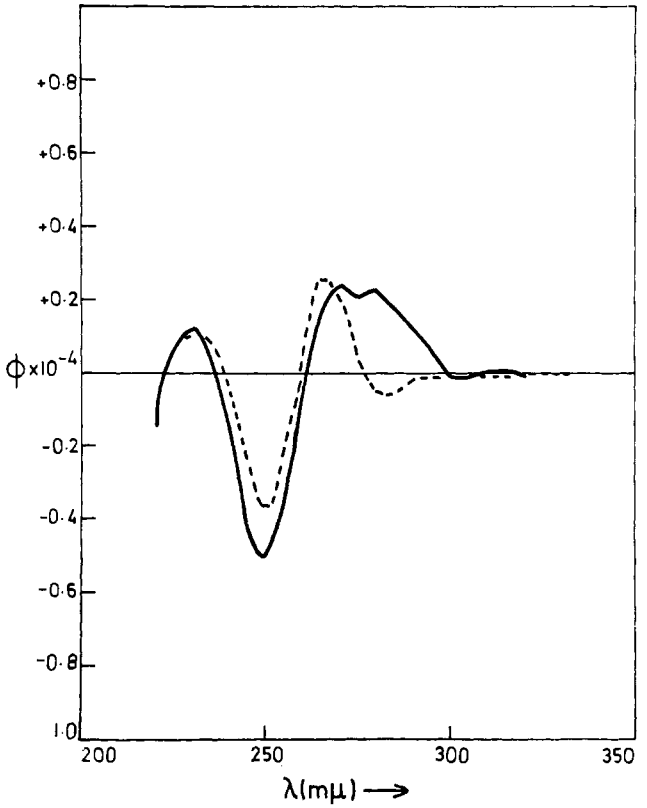

FIGURE 8: Optical rotatory dispersion of 3′–5′-GpGpG in 10^{-1} M Tris-HCl (pH 7) at room temperature. Concentration 10^{-4} M, measured (——) and calculated from eq 4 (— —).

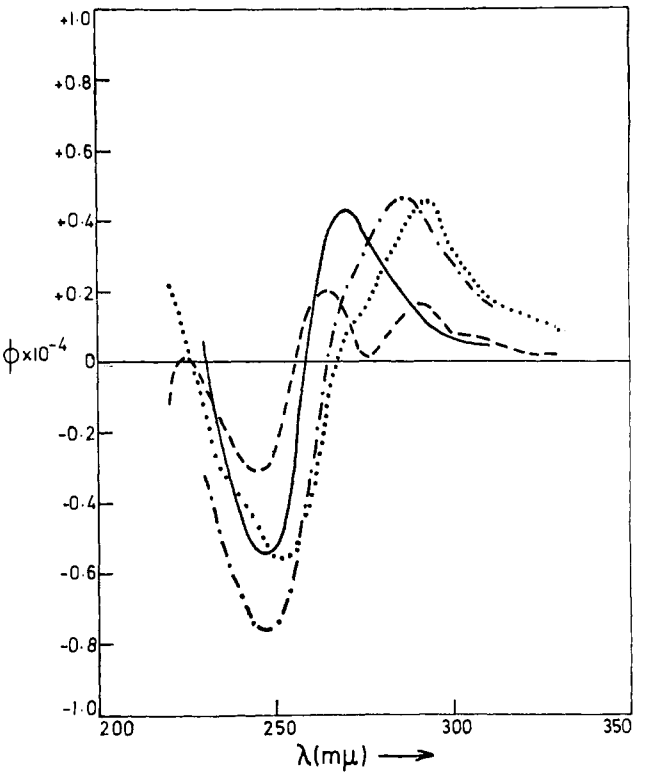

FIGURE 9: Optical rotatory dispersion of GpGpU in phosphate buffer measured (——) and calculated from eq 4 (— —). Optical rotatory dispersion of GpGpC in phosphate buffer, measured (— · —), and calculated from eq 4 (· · · ·).

and tetramer, rich in guanylic acid and compare them to those predicted from nearest-neighbor approximation (Cantor and Tinoco, 1965). As mentioned above, guanine-rich oligonucleotides form self-associated complexes, presumably *via* H-bond formation. In order to ensure that the measured optical rotatory dispersion to that of monomeric oligonucleotides, dilute solutions ($\sim 10^{-4}$ M) were preheated at above their transition temperature for 2–3 hr to cause disaggregation and were then brought back to desired temperature of measurement. That no degradation took place upon heating was checked by measuring optical rotatory dispersion of the solution either after the solution being aged for a longer time at low temperature (4–5°) or being frozen for a short time. This was further confirmed by molecular weight determination.

Figures 6 and 7 show the measured optical rotatory dispersion of melted 2′–5′-GpGpGp and -GpGpGpGp at room temperature and those of computed from eq 4 and 5 for tri- and tetramer, respectively. Earlier, rotation of dinucleoside mono-

$$\phi_{ijk} = \frac{2\phi_{ij} + 2\phi_{jk} - \phi_j}{3} \quad (4)$$

$$\phi_{ijkl} = \frac{2\phi_{ij} + 2\phi_{jk} + 2\phi_{kl} - \phi_j - \phi_k}{4} \quad (5)$$

phosphates was used to compute the rotation of oligomer and polymer (Cantor and Tinoco, 1965; Cantor *et al.*, 1966). Assumption was, however, made that terminal phosphate group does not have any effect on optical properties. This was however not found to be the case. Since optical rotatory dispersion of GpGp is strikingly different from that of GpG, we have computed rotation of GpGpGp and GpGpGpGp using rotation of GpG and GpGp as well (shown in Figures 6 and 7). Neither is found to be as satisfactory as in other cases (Cantor and Tinoco, 1965; Inoue *et al.*, 1967).

Figure 8 shows the measured optical rotatory dispersion of 3′–5′ GpGpG and Figures 9 and 10 compare the measured optical rotatory dispersion of GpGpC and GpGpU (both measured earlier by Cantor and Tinoco, 1965) and monomeric GpGpGpU with those calculated from the nearest-neighbor approximation. Evidently the agreement between calculated and the measured optical rotatory dispersion spectra is less satisfactory than those reported for oligonucleotides obtained from T_1 and pancreatic ribonuclease digest of RNA (Cantor and Tinoco, 1965; Inoue *et al.*, 1967). In most cases the detailed shapes of the optical rotatory dispersion curves were more or less accurately predicted.

The melting of single-stranded polynucleotide helices is nearly noncooperative (Van Holde *et al.*, 1965). This means that the nearest-neighbor approximation should be valid to a great extent. Evidently this is in contrast to those which have been found for guanine-rich oligonucleotides. The question is why it is so. As a tentative explanation the following could be mentioned. Both theoretical calculations and experimental observations (Jordon and Pullman, 1968; Hart and Davis, 1969) suggest that guanine could exist in both syn and anti conformations. It is probable that energy difference between two conformers is bit higher than that of adenosine, which is of the order of kT (Chan and Nelson, 1969). This could probably explain why optical rotatory dispersion of ApA and ApAp resemble each other (Davis, 1965), in contrast optical rotatory dispersion of 2′–5′-GpG and -GpGp are very much different. The maximum difference is observed around 260

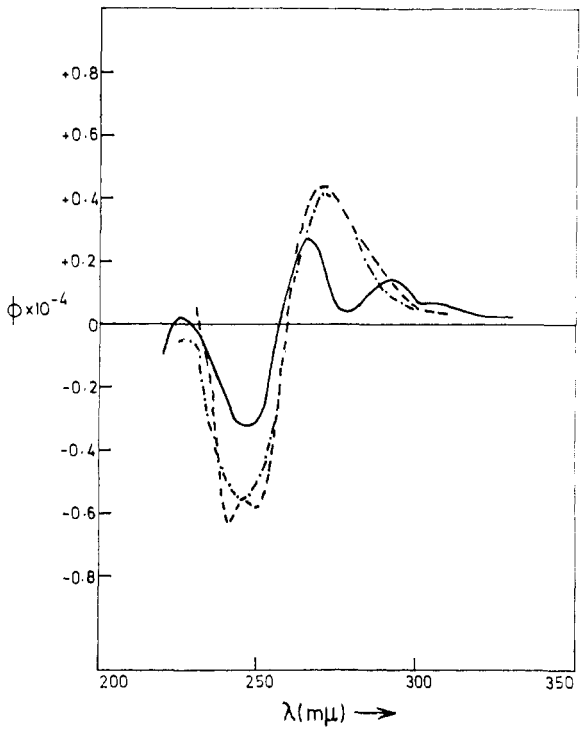

FIGURE 10: Optical rotatory dispersion of melted GpGpGpU in 0.1 M phosphate–0.5 M NaCl, (—·—) measured; calculated from eq 4 (——) with measured GpU and GpG data, (— —) with calculated GpU and GpG data.

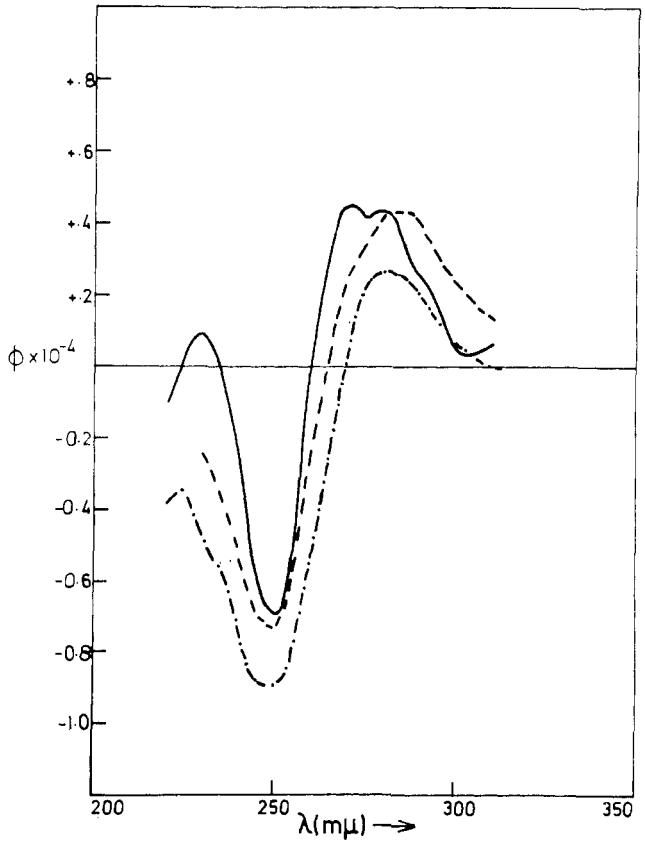

FIGURE 11: Optical rotatory dispersion of melted GpGpGpC in 0.1 M phosphate buffer (pH 7); measured (—·—); calculated from eq 4 (— —); predicted optical rotatory dispersion of poly(G) (——).

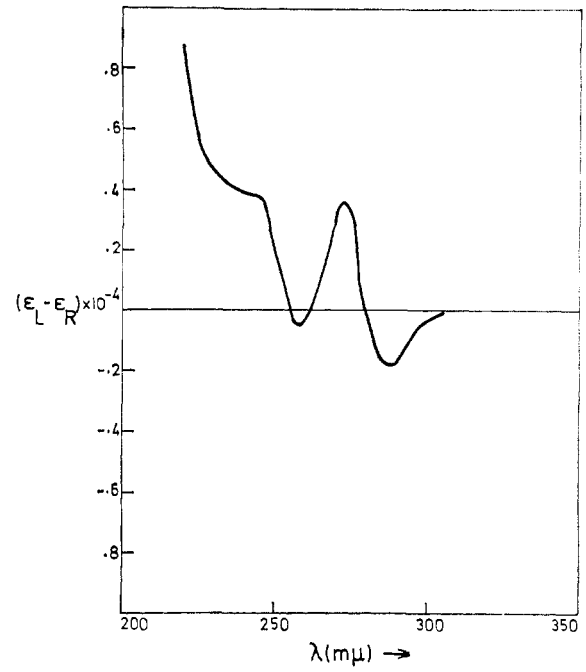

FIGURE 12: Circular dichroism of 2′–5′-GpG in 10^{-2} M Tris-HCl (pH 7). Concentration 7×10^{-5} M.

mμ, where B_{2u} transition occurs (Clark and Tinoco, 1965). In case of trimer and tetramer and so on, rotation around a single bond will be restricted differently in each case. This would mean that the relative proportion of conformers would depend on the chain length. If this is to be the case, one would not expect an agreement between measured optical rotatory dispersion and those computed from the nearest-neighbor approximation. On the other hand, if optical rotatory dispersion of tetramers are computed from eq 5 using calculated value of dimer, a better agreement is expected. The optical rotatory dispersion of GpG is first calculated from the measured optical rotatory dispersion of 3′–5′-GpGpG according to eq 4 and is then used to calculate optical rotatory dispersion of GpC and GpU from the measured values of GpGpC and GpGpU, respectively. With these calculated values of dimer the optical rotatory dispersion of GpGpGpC and GpGpGpU are computed and compared to the measured ones (Figures 10 and 11). Similarly the optical rotatory dispersion of the single-stranded poly(G) is predicted from the calculated optical rotatory dispersion of GpG and is shown also in Figure 11. Surprisingly it resembles quite closely (except in magnitude) that of the structured poly(G) (Ulbricht *et al.*, 1966). The agreement is certainly better and thereby suggests itself the existence of syn–anti equilibria in oligoguanylic acids. In a recent paper (which appeared when this work was completed) Brahms *et al.* (1969) came to a similar conclusion.

One additional observation deserves some comments. In both 2′–5′- and 3′–5′-GpC longest wavelength troughs are relatively insensitive to pH, temperature, and solvent perturbation. It is also comparatively intense. For better confirmation circular dichroic spectra of 2′–5′-GpG is shown in Figure 12. The end phosphate group does not seem to have effect on the magnitude of long-wavelength trough (Figure 12). In contrast, the cotton effect between 230 and 250 mμ is very sensitive to such changes. Furthermore in 2′–5′ oligomers characteristic long-wavelength trough of dimer is retained but in optical rotatory dispersion of 3′–5′-GpGpG

(Figure 8) and of predicted optical rotatory dispersion spectra of poly(G) (Figure 11) a new band appears. Recent work of Bush and Scheraga (1969) on monomer, oligomer, and polymer of adenylic acids suggests that long-wavelength band which is present only in oligomers longer than dinucleotide, is due to nonconservative $n-\pi^*$ transition and not the positive component of the exciton splitting of $\pi-\pi^*$ transition. For this reason, a long-wavelength band is not seen in monomer and dimer. This suggestion is, however, not compatible with the observed differences in optical rotatory dispersion spectra of 2′–5′ and 3′–5′ oligomer of guanylic acids, longer than dimer. *A priori* one would not assume that the interior guanine residue of 2′–5′ and 3′–5′ oligomers would likely to be solvated differently with respect to its counterpart in monomer and dimer. In conclusion, it seems fair to say that the optical properties of 3′–5′-oligoguanylic acids can be predicted if the next-nearest-neighbor interactions are introduced empirically. The role of 2′-OH group on polynucleotide structure is not clear. A comparative study of solvent effect on the conformation of 2′–5′ and 3′–5′ oligomers might be interesting and useful in explaining how 2′-OH group participates into introducing extra stability of helical conformation of 3′–5′-polynucleotide chain.

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Addendum

Here it is worthwhile to mention, although equilibrium ultracentrifuge data clearly indicated that $(Gp)_3$ and $(Gp)_4$ were not self-associated under the conditions of optical rotatory dispersion measurements, optical rotatory dispersion of $(Gp)_3$ and $(Gp)_4$ were not quite reproducible under slight variation of salt concentration. The magnitude of long-wavelength peak was found to vary only slightly but trough intensity changes considerably. The accurate determination of these small changes was difficult due to low signal-to-noise ratio. Nevertheless such changes were not large enough to account for the difference between the calculated and the observed optical rotatory dispersion data even qualitatively.

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