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*POLYNUCLEOTIDES, VI. INTERACTION BETWEEN POLYGUANYLIC ACID AND POLYCYTIDYLIC ACID**

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Although a number of studies on the interaction between poly dG and poly dC have been reported,[1–3] as well as observations on hybrids formed between the ribopolynucleotides and the deoxyribopolymers,[4] little definitive work has appeared on complex formation in reactions between polyguanylic and polycytidylic acid. The urgency of studies of these reactions is self-evident in view of the crucial contributions of the G-C base pair to the structure of RNA. It has been claimed that such studies are very difficult to carry out with synthetic homopolymers because of the occurrence of stable self-bonded guanine structures.[5] This argument is hardly admissible in view of the easy displacement of one polymer from a complex by another.[6–8] Complex formation between poly C and oligoguanylic acids has been described,[9, 10] and details of the interaction of poly C and poly G have been reportedly in press[11] in *Journal of the American Chemical Society* since 1963 but are as yet unavailable.

Development of a technique for the enzymic polymerization of GDP by the action of *E. coli* polynucleotide phosphorylase[12] has recently made available for the first time a clean product which can reasonably be called polyguanylic acid. An earlier description[13] of the preparation of poly G gave only very short polynucleotides in our hands. Another approach to the preparation of poly G + poly C involves an enzymic synthesis of poly G in the presence of poly C using GTP and RNA polymerase.[14] However, the immediate product is treated with ribonuclease to remove excess of poly C. It is not unlikely that even in the double-stranded com-

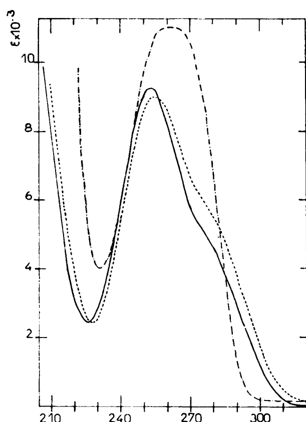


FIG. 1.—Spectra of poly G in water at pH 6 ——, at pH 1.18 ------, and at pH 12.15 -·-·-.

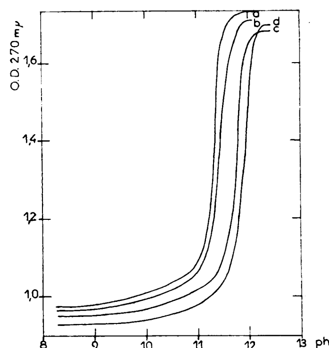


FIG. 2.—Alkaline titration of poly G at 270 mμ in (*a*) 0.25 *M* NaCl, (*b*) 0.15 *M* NaCl, (*c*) 0.05 *M* NaCl, and (*d*) 0.01 *M* NaCl., Apparent pK = 11.32, 11.43, 11.76, and 11.86, respectively.

plex some cleavage of poly C occurs without dissociation of nucleotide material from the complex. In this paper we will describe properties of poly G and its interaction with poly C.

Materials and Methods.—Polyguanylic acid: Poly G was synthesized from GDP by the procedure described by Thang *et al.*[12] The incubation mixture was treated with sufficient phenol to cause slight turbidity, clarified by centrifugation, and then dialyzed successively against 0.2 *M* NaCl containing 0.01 *M* Tris pH 8 and 0.001 *M* EDTA, then water at 0,° and finally lyophilized. S_{20} = 9.54 in 0.1 *M* NaCl 0.05 *M* cacodylate pH 7.0. Alkaline hydrolysis and estimation of end group nucleoside and nucleoside-2′(3′), 5′ diphosphate showed average chain lengths to be greater than 100. Owing to the ready aggregation of oligo G, a chemical estimation of molecular size is of more significance than that based on the physical approach. The polymer dissolved readily in water to give nonviscous solutions even at concentrations of 7 mg/ml.

Spectrophotometric titrations: Titrations were performed at a suitable wavelength in a Zeiss spectrophotometer, the titration being carried out directly in the cell with constant agitation by means of a magnetic stirrer.

Thermal dissociation curves: These were performed in a Cary 14 spectrophotometer equipped with a thermostated cell holder. Temperature was increased linearly by about 1 degree per minute. Temperature inside the cell was obtained by means of a small thermocouple.

Results and Discussion.—Spectra of poly G in water at pH 6, 1.18, and 12.15 are shown in Figure 1. In accord with results obtained earlier with chemically synthesized oligoguanylic acids,[15] at neutral pH there is very marked hyperchromicity, somewhat less at acidic pH, and none at all at alkaline pH, contrary to statements by Schramm.[16] As in the case of the oligoguanylics, a very marked shift in alkaline pK value relative to that of GMP can be seen. Spectrophotometric titrations of poly G in sodium chloride solutions of different molarity are shown in Figure 2. It can be seen that increase in salt concentration reverses the shift in pK value (for a discussion of this effect see ref. 17, pp. 456 and 518). Whereas the alkaline titra-

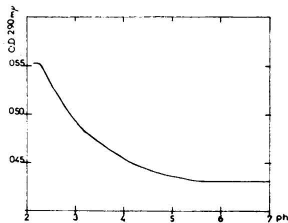


FIG. 3.—Acidic titration of poly G in 0.1 *M* NaCl at 290 mμ. Apparent pK = 3.0.

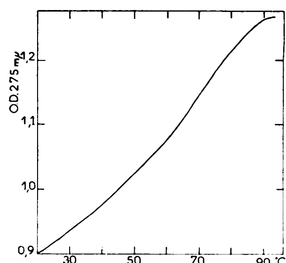


FIG. 4.—Temperature-ultraviolet absorption profile for poly G at 275 mμ in water in the absence of salt.

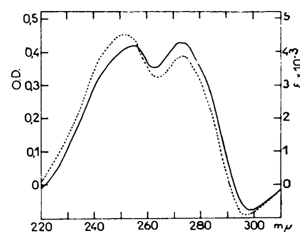
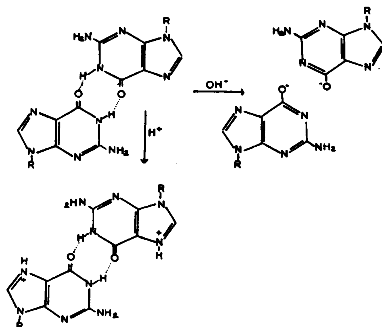


FIG. 5.—Difference spectra of poly G, structured and nonstructured, in water (at 20° and 95°)——; and of guanosine and poly G in water at pH 6 - - - -.

tion of poly G is abrupt and indicative of a cooperative dissociation, acidic titration (Fig. 3) appears to be noncooperative though the base pK value is increased relative to that of GMP. It thus appears that whereas removal of protons leads to an abrupt collapse of secondary structure, addition of protons does not interfere seriously.

Although the number of strands and the exact arrangement of the secondary structure of poly G is unknown, the two processes can be represented as shown in the scheme. Regardless of whether addition of a proton occurs at N7 or at the



extranuclear-2-amino group, hydrogen bonding is not destroyed until sufficient bases carry a positive charge. Indeed, there is no definitive evidence that protonation of all the guanine residues does, in fact, lead to destruction of secondary structure despite charge repulsion effects. (This point will be discussed further in a report on the properties of poly N7-methylguanylic acid.[18]) There is no experimental evidence to support the suggestions that hydrogen bonds between N7 and the 2-amino group are present or that thermal stability indicates more than two polynucleotide strands.[19] In fact, poly G does have an extremely stable structure and shows no hypochromic change up to 100° in 0.002 *M* Na^+. Thermal denaturation of poly G in water in the complete absence of added salt is shown in Figure 4. Even in water at pH 6, thermal hyperchromic effects are complete only above 90°. Since even traces of magnesium ions are absent, the profile is extremely broad, as is the case for poly A + poly U at very low ionic strength. Difference spectra between ordered and disordered poly G obtained directly in this way are given in Figure 5, as are difference spectra between GMP and poly G in water at pH 6. The two are

FIG. 6.—Mixing curve of poly G + poly C in 0.1 M NaCl containing 0.05 M sodium cacodylate pH 7.0 (a) at 280 mμ, (b) at 245 mμ, and (c) at 262 mμ.

significantly different since the latter does not take into account any residual hypochromicity in a disordered poly G strand.

Poly G interacts rapidly with poly C to give a stable 1:1 complex. This result is in disagreement with that reported previously.[10] The mixing curve of the two polymers in 0.1 M NaCl 0.05 M sodium cacodylate pH 7 is shown in Figure 6. Spectral effects at λ245, λ262, and λ280 mμ all indicate a 1:1 complex. The relative hyper- and hypochromicities cannot be related to difference spectra between (poly G + poly C) and the two polymers in methanol because the two reactions are different:

$$\text{Poly } G_{\text{struc.}} + \text{poly C} \rightarrow (\text{poly G} + \text{poly C}) \rightarrow \text{poly } G_{\text{nonstruc.}} + \text{poly C}.$$

This ratio is maintained and no tendency to form complexes with a ratio 2G:1C has been observed, even after long periods of time at 25°, after heating to 100° for 3 min, or after addition of magnesium ions ($10^{-3}M$). However, when poly G was treated with alkali to cause partial degradation to oligo G and short-chain poly G, mixing curves showed the ready formation of complexes containing 2G:1C (Fig. 7). This suggests that the enzymatically synthesized preparations of poly G, reported previously,[13] were in fact not only polydisperse but of relatively short chain length.

Since the complex poly G + poly C is extremely stable, the thermal ultraviolet absorption profile (Fig. 8) was obtained at very low salt concentration and in 80 per cent methanol. In 50 per cent methanol no spectral effects were observed up to 100°. Fresco and Hirschbein[14] have described the thermal denaturation of a complex between poly G and poly C prepared by RNA polymerase from poly C and GTP

FIG. 7.—Mixing curve of oligo G + poly C in 0.1 M NaCl containing 0.05 M sodium cacodylate pH 7.0 (a) at 280 mμ, (b) at 245 mμ, and (c) at 262 mμ.

FIG. 8.—Temperature-ultraviolet absorption profile at 280 mμ for poly (G + C) in 80% methanol containing 0.001 M NaCl and 0.0005 M sodium cacodylate pH 7.0. The T_m is approximately 89° and the process is irreversible. The effect of temperature on poly (G + C) in 50% methanol (- - - -) is also shown.

FIG. 9.—Thermal-ultraviolet absorption profile of poly C + 2 oligo G at 280 mμ in 50% methanol containing 0.1 M NaCl and 0.01 M sodium cacodylate pH 7.0. The thermal denaturation is completely reversible in this solvent. In 80% methanol the complex is dissociated at 20°.

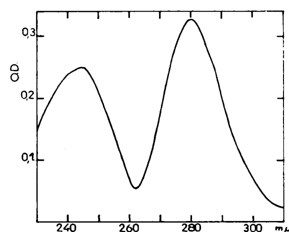


FIG. 10.—Difference spectra between poly (G + C) and poly G + poly C in 80% methanol containing 0.001 M NaCl and 0.0005 M sodium cacodylate pH 7.0.

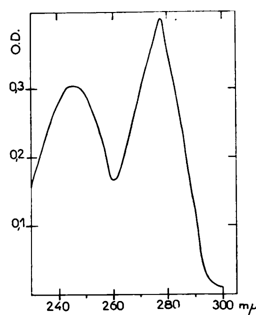


FIG. 11.—Difference spectra between poly C + 2 oligo G complexed and noncomplexed in 50% methanol containing 0.1 M NaCl and 0.01 M sodium cacodylate pH 7.0.

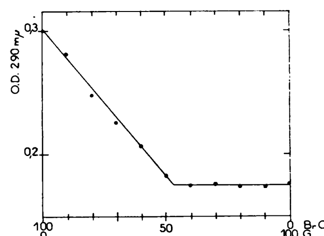


FIG. 12.—Mixing curve at 290 mμ of poly G + poly BrC in 0.1 M NaCl containing 0.05 M sodium cacodylate pH 7.0.

followed by RNAase treatment, in 50 per cent methanol and the same salt concentration. Possibly their material contained short chains. Whereas poly G, (poly G + poly C), and (poly G + poly BrC) are all resistant to venom diesterase, preliminary results indicate that the latter two complexes may be slowly attacked by pancreatic RNAase with random cleavage of the poly C chain without dissociation of the complex, so that there is a complete absence of optical effects.

The temperature ultraviolet absorption profile of the complex (oligo G + poly C) in the ratio 2:1 in 50 per cent methanol and low salt is shown in Figure 9. Difference spectra, corrected for expansion, of (poly G + poly C) structured and denatured in 80 per cent methanol and of (poly C:2 oligo G) in 50 per cent methanol are shown in Figures 10 and 11.

Although polyinosinic acid complexes with poly A, poly G does not do so under a variety of conditions examined. However, interaction occurs readily with poly 5-bromocytidylic acid.[20] Again, the ratio of nucleotides is G:BrC = 1:1 and this does not change after a week at room temperature. No indications of a stoichiometry 1G:2BrC, or 2G:1BrC, have been obtained (Fig. 12). The increased stability of poly (G + BrC) relative to poly (G + C) is readily shown by the alkaline spectrophotometric titration of the complexes. Whereas GMP has a pK = 9.16 and poly G (in 0.15 M NaCl) has a pK = 11.43, poly (G + C) shows a pK of cooperative dissociation (Fig. 13) of 12.27 in 0.15 M NaCl. In the case of poly (G + BrC), this is shifted to 12.56. Under the same conditions in 80 per cent methanol as used for thermal denaturation of poly (G + C), no spectral effects could be observed for poly (G + BrC) up to 100°. In 80 per cent methanol containing 0.0015 M Na^+ and 4 per cent ethylene glycol, poly (G + BrC) shows indications of denaturation above 90° but the T_m is above 100°.

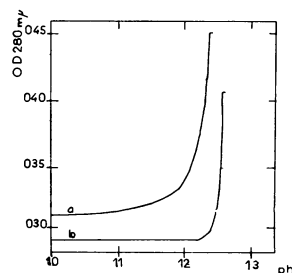


FIG. 13.—Alkaline titration of (a) poly (G + C) and (b) poly (G + BrC) at 280 mμ in 0.15 M NaCl. Apparent pK of dissociation poly (G + C) = 12.27; poly (G + BrC) = 12.56.

Summary.—The physical properties of high-molecular-weight poly G are described. While interaction

with poly C gives a very stable 1:1 complex, no indication of complex formation with a stoichiometry of 2G:1C, or 1G:2C, is observed. However, degraded poly G readily forms a complex 2G:1C. Poly G and poly BrC also form a 1:1 complex which is even more stable than that between poly G and poly C.

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*THE CHEMICAL NATURE OF PHOTOREACTIVABLE LESIONS IN DNA**

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Ultraviolet-inactivated transforming DNA can be reactivated by photoreactivating enzyme from yeast in the presence of visible light.[1] The ability of ultraviolet-irradiated polydeoxynucleotides to compete with ultraviolet-inactivated transforming DNA for yeast photoreactivating enzyme provides a method of determining the effect of the enzyme plus photoreactivating light on compounds which themselves are lacking in biological activity. A synthetic polymer may be said to be photoreactivable if (1) it competes, and (2) its ability to compete is reduced by treating the irradiated polymer with enzyme plus light before addition of the transforming DNA, as shown by Rupert for poly dG:dC but not for poly d(AT): d(AT).[2]

This communication describes the photoreactivability of a wider range of simple polydeoxynucleotides of known composition and sequence, and includes experiments on competition by several DNA's of varying base composition. The homopolymer