Complex Formation between Polycytidylic Acid and Guanine Oligonucleotides

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A study of the characteristics of the guanine-cytosine bond is important to the understanding of its contributions to the secondary structure of nucleic acids. However, such a study is very difficult to carry out with synthetic homopolymers because of the occurrence of stable self-bonded guanine structures (1). It is almost impossible to obtain polyriboguanylic acid (poly G) in a single-stranded form at reasonable conditions of temperature and pH (2), but there are a few reports concerning interactions between it and poly C. Radding, Josse, and Kornberg (3) obtained poly dGdC mixtures with variable G:C ratios as products of deoxyribonucleic acid polymerase, and Fresco (4) has reported the occurrence of polyriboguanine (G + C) as an intermediate in G + G + C formation.

If guanine oligonucleotides are used in place of poly G, however, the interference caused by self-bonding of guanine residues becomes less troublesome and more easily controlled. A preliminary study of the interaction of GpGpG with poly C has been reported (5). A complex containing equal amounts of guanine and cytosine residues (G + C) was observed; it was stable at room temperature. At low temperatures, another, less stable complex (G + G + C) consisting of two parts of guanine and one of cytosine was formed. The present report extends these observations to other guanine oligonucleotides and also 

Concentrations of polymers are expressed as equivalents of nucleoside base, calculated from measurements of inorganic and total phosphate. Difference spectra are calculated from the difference in absorbance at temperatures above and below the transition. These differences are transformed to molar extinction coefficients by dividing the absorbance of 1 cm of solution by the total base concentration of the solution, expressed as moles per liter.

Thermal dissociation of complexes was carried out as previously described (9). The pH titration of poly C was performed in an automatic titrator (Radiometer, Type TTT1c, Copenhagen), with the use of 0.01 n HCl in the titrating syringe and 20 ml of poly C, stirred with a stream of CO2-free air, in the reaction vessel. Samples of 0.5 ml were removed to glass-stoppered cuvettes for measurements of absorption spectra in a Cary model 14 spectrophotometer.

Properties of G + C—The fact that the G—C bond forms readily upon mixing solutions of poly C and guanine oligonucleotides makes it possible to determine minima in mixing curves under conditions in which G + G self-bonding occurs slowly. Fig. 1 shows such a mixing curve for poly C + GpG. The G—C minimum at the 0.5-mole fraction is seen within a few hours after mixing the solutions. The downward curve of the guanine-rich side, which represents self-aggregation of the excess GpG, develops more slowly and is obviously concentration-dependent. The right side of the curve was not observed to become linear even after the solutions had been maintained at 3° for up to 6 weeks.

Effects of Nucleotide and Salt Concentrations—An increase in the nucleotide concentration markedly stabilizes the G—C bond. At pH 7.4, the Tm for (poly C + GpG) increased from 14.0° to 22.5° as the base concentration was raised from 0.06 mM to 0.75 mM.

An increased salt concentration lowers the stability of the GC complex at both pH 6.2 and pH 7.4 (Table I). This reaction may be an effect of the salt on poly C, and it will be discussed in the section on (C + G + C).

Effect of Terminal Phosphate Group—At pH 6.2, the presence of a terminal phosphate group on guanine dinucleotide has a pronounced effect upon the stability of the G + C complex formed (Table II). Thus, while the G + C complex formed with GpG in Fig. 2 had a Tm of 25.5°, the complex formed under the same conditions with GpGp had a Tm of 16°. This effect of the terminal phosphate group falls off quickly with an increasing chain length, for the Tm values for the G + C complexes formed by poly C with GpGpG and GpGpGp under the same conditions are 45.6° and 45.0°, respectively.

**EXPERIMENTAL PROCEDURE**

Poly C was prepared with polynucleotide phosphorylase from *Azotobacter vinelandii* by published procedures (6). Guanine oligonucleotides were prepared by polymerization of guanosine-2',3'-cyclic phosphate with Taka-Diastase T1 ribonuclease and were separated on a DEAE-urea column, as described elsewhere (7). Dephosphorylation of the oligonucleotides was accomplished with the use of *E. coli* alkaline phosphatase and subsequent purification on DEAE. GpGpUp was obtained from a pancreatic ribonuclease digest of a copolymer containing both guanylic and uridylic acid (7), and 2',5'-GpG from digestion of chemically synthesized poly G (for which we are indebted to Dr. A. M. Michelson) with T1 ribonuclease (8). Sodium cacodylate was obtained from Mallinckrodt Chemical Works, St. Louis.

1 The abbreviations used are: poly G, polyriboguanylic acid; and poly C, polyribocytidylic acid.
2 We have been informed that this complex has also been observed by J. R. Fresco, J. Massonlić, and R. D. Blake.
The difference spectra for the melting of (poly C + GpG) and (poly C + GpGp) at pH 6.2 are shown in Fig. 2. GpGp does not react with poly C at pH 7. From pH 7.2 to 9, the difference spectrum for (poly C + GpG) is slightly different from that for the acid form, in that the two peaks are more nearly equivalent in height and the trough at 260 mμ is lower.

A plot of \( T_m \) against \( 1/n \) for the poly C + (Gp)n series at pH 6.2, although it has only 3 points, gives a value of 103-105°.

**TABLE I**

Effect of salt concentration upon \( T_m \) of poly C + GpG

<table>
<thead>
<tr>
<th>pH</th>
<th>NaCl concentration</th>
<th>( T_m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2</td>
<td>1.0</td>
<td>36.1</td>
</tr>
<tr>
<td>7.4</td>
<td>0.2</td>
<td>25.4</td>
</tr>
</tbody>
</table>

**TABLE II**

\( T_m \) values for complexes between guanine oligonucleotides and poly C

Except where noted, all values were determined in solutions containing 0.2 M NaCl-0.002 M cacodylate buffer, pH 6.2, and 0.7 to 0.9 mM base.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Type of complex</th>
<th>( G + C )</th>
<th>( G + G + C )</th>
<th>( C + G + C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>GpG</td>
<td></td>
<td>25.5</td>
<td>Not observed</td>
<td>27.1*</td>
</tr>
<tr>
<td>GpGp</td>
<td></td>
<td>16.0</td>
<td>25.5, 45.6</td>
<td>42.2†</td>
</tr>
<tr>
<td>GpGpG</td>
<td></td>
<td>45.6</td>
<td>24.7, 45.0</td>
<td></td>
</tr>
<tr>
<td>GpGpGp</td>
<td></td>
<td>58.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GpGpGpG</td>
<td></td>
<td>45.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GpGpGpGp</td>
<td></td>
<td>58.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GpGpGpGpG</td>
<td></td>
<td>58.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2',5'GpGpGpGpGp</td>
<td></td>
<td>19.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The base concentration used was 4.32 mM. The \( T_m \) for GpGp alone at this concentration is 22.3°.
† The base concentration used was 0.063 mM.
‡ See the text for conditions.

**Fig. 1.** Interaction of poly C and GpG. Conditions: 0.8 mM base and 0.2 M NaCl 0.005 M sodium cacodylate buffer, pH 6.2, reacted for 3 days at 3° and read at 3° in cuvettes with 1-mm light paths. Optical densities of starting solutions at room temperature are indicated by △.

**Fig. 2.** Spectral changes on dissociation of complexes of poly C with GpG and GpGp. The conditions were as in Fig. 1. —, poly C and GpG, guanine mole fraction = 0.50; --, poly C + GpGp, guanine mole fraction = 0.50.

**Fig. 3.** Relationship between dissociation temperature and reciprocal chain length of guanine oligonucleotide bonded to poly C in (G + C) complex. The pH is 6.2 in standard solvent, and the nucleotide concentration is 0.8 mM.† In this and following experiments, it must be remembered that difference spectra reflect the relative changes in \( e \) with wavelength, but not necessarily the exact magnitude of the change at any given wavelength. In none of these experiments is there any assessment of the completeness of the G-C interaction, save that mixing curves in general had straight sides. Even this must be qualified in the cases where there was simultaneous formation of G + G aggregates and concomitant curvature of the side of the mixing curve containing predominantly guanine residues.
for the $T_m$ of poly C + poly G of infinite length (Fig. 3). This value is in good agreement with the $T_m$ of 110° calculated from the Marmur and Doty equation (10) for poly dC + poly dG at almost the same sodium ion concentration.

**Properties of G + G + C**—When solutions of poly C and a guanine oligonucleotide are mixed in varying proportions and stored for a sufficiently long period of time at 3°, a complex containing 2 guanine residues for each cytosine residue is formed, as evidenced by a minimal absorption at the 0.67-mole fraction of guanine. A preliminary study of this G + G + C complex (5) has indicated that the second strand of guanines may be removed without disrupting the two-stranded G + C core of the complex. The bonds holding this second strand of guanines are considerably weaker than the G + C bond, as evidenced in the system (poly C + 2 GpGpG) by the fact that the $T_m$ value of 24.7° was found for the transition

$$\text{(G + G + C)} \rightarrow \text{(G + C) + G}$$

and 45° for the transition

$$\text{(G + C) \rightarrow G + C}$$

(Conditions: 0.2 M NaCl-0.002 M cacodylate buffer, pH 6.2; 6.3 x $10^{-4}$ M base.) The first transition was extremely time-dependent, and dependence on time has been found to be a characteristic of the melting of self-bonded guanylate structures (9). The spectral changes associated with this first transition were also very similar to the difference spectrum observed during the melting of self-bonded GpGpGp.

The biphasic nature of this interaction makes it possible to obtain a G + C complex with any given guanine oligonucleotide, since one need only choose conditions for the aggregation that minimize the formation of the G + G bond, and therefore the G + G + C complex, for that oligonucleotide. This can be accomplished by selecting an incubation temperature above the melting temperature of the self-bonded guanylate aggregate or by working at base concentrations at which the formation of the G + G bond is so slow as to offer little complication to the study of G + C. For example, in the case of GpGpG, the G + G + C complex is not formed until the base concentration is raised to $10^{-3}$ M. This level is 20 times that required to demonstrate the G + G + C complex with GpGpG.

**Properties of C + G + C**—A third type of complex containing two parts of cytosine residues to one part of guanine moieties (C + G + C) has also been demonstrated (Fig. 4). Preliminary experiments on this interaction indicated a critical effect of pH in the region of pH 5 to 6. No group titrating in this region was present in the guanine oligonucleotides we were using, which lacked a terminal phosphate. Therefore the degree of protonation of poly C, which occurs in this range, seemed important in the formation of C + G + C. Consequently the titration of poly C was investigated in some detail.

A solution of poly C containing 0.05 mM base in 0.2 M KCl, pH 7.35, was titrated with 0.01 M HCl, and the spectral changes were followed at 25°. The optical density ratio, $D_{280}:D_{215}$, was used as a measure of the protonation in order to obviate corrections for slight volume changes occurring as a result of the acid addition. Fig. 5A shows the change in this ratio with pH. Since salt concentration influences the position of the titration point (11), all measurements were made in 0.2 M KCl to correspond to our usual salt conditions in this series. The midpoint of the titration under these conditions occurs at pH 5.55.

![Fig. 1. Formation of a C + G + C complex between poly C and GpGpG. Conditions: 0.063 mM base and 0.2 mM NaCl-0.002 mM sodium cacodylate buffer, pH 6.2, reacted for 24 hours at room temperature.](image)

**Fig. 5.** Spectral changes in poly C. A, effect of pH; B, effect of temperature. See the text for details.

**TABLE III**

<table>
<thead>
<tr>
<th>Complex</th>
<th>Melting</th>
<th>Dissociation rate</th>
<th>pH range</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>G + C</td>
<td>Monophasic</td>
<td>Rapid</td>
<td>Broad*</td>
<td>Marked concentration dependence†</td>
</tr>
<tr>
<td>G + G + C</td>
<td>Biphase</td>
<td>Slow</td>
<td>Narrow, ca. pH 5-6</td>
<td>Requires protonation of half of cytosine residues</td>
</tr>
<tr>
<td>C + G + C</td>
<td>Monophasic</td>
<td>Rapid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The complex may be formed over the approximate pH range of 6 to 11. The lower limit is fixed by the protonation of the cytosine moiety and is influenced by the salt concentration.
† When guanine oligonucleotides are used, the G-G bond in this complex is strongly concentration-dependent. Whether this would be true for poly G is uncertain.
The effect of temperature on the protonation of poly C was also investigated. As the temperature is raised, there is a very slow deprotonation, as evidenced by a drop in the $D_{280}/D_{260}$ ratio (Fig. 5B) and a hypochromic shift in the maximum. Under our conditions (0.2 M KCl-0.01 M acetate buffer, pH 5.80; 0.053 mm base), the deprotonation is complete at about 26°C. Equilibration at each temperature takes up to 12 hours. The change in the pH of the buffer on heating over this range is very slight, since the $\Delta H^o$ for acetic acid is very low (12).

A series of mixing curves was set up with poly C and GpG at pH 5.0, 5.8, and 6.0 (0.06 mm base in 0.2 M KCl-0.01 M acetate buffer). After 18 hours at 3°C, the mixing curve at pH 5.8 shows the formation of $C + G + C$ with a 31% hypochromicity. At pH 5.0, the curve is not straight-sided even after several days, although the minimum appears to be near that for $C + G + C$ in that it has about 10% hypochromicity at that point. The pH 6.0 series shows a 1:1 $G + C$ complex, but no evidence of $C + G + C$ formation. There is no complicating GpG interaction at these concentrations. Therefore it appears that the $C + G + C$ complex is formed most completely and most rapidly at the point at which the poly C is partially protonated. The characteristics of the three types of G-C complexes are presented in Table III.

The difference spectrum for the $C + G + C$ complex of poly C and GpGpG is seen in Fig. 6, along with the monophasic

![Fig. 6. Properties of the $C + G + C$ complex containing poly C and GpGpG. The conditions were as in Fig. 4. A, difference spectrum; B, melting curve.](image)

![Fig. 7. Difference spectra for interaction of poly C with 2',5'-GpG and with GpGpUp. See the text for details. ---, poly C + 2',5'-GpG, guanine mole fraction = 0.50; -- --, poly C + GpGpUp, [G]/[G] + [C] = 0.50.](image)

![Fig. 8. Possible configurations for $G + G + C$ and $C + G + C$.](image)
melting curve of this complex. The $T_m$ of 42.2° is very close to the $T_m$ of 43° observed in the second phase of G + G + C melting, i.e., the breaking of the G + C complex, at this concentration. The melting of G + C + G shows none of the sluggish characteristics of either G + G or C + C bondings; optical equilibrium is rapidly reached, and formation of the complex itself occurs as rapidly as the formation of the G + C complex and much faster than the formation of G + G + C.

Miscellaneous Interactions

Interaction of Poly C and GpGpUp—Previous studies with the adenine-uracil system (13, 14) had revealed that noncomplementary bases in one strand weaken the interaction between the two strands, even though the noncomplementary bases evidently do not occupy a bonding space in the helix, but dangle or loop out away from the bonding pairs which comprise it. It was therefore of interest to investigate bonding with a guanine oligonucleotide containing a noncomplementary base, such as GpGpUp.

Poly C was interacted with GpGpUp in the proportions 1C:1G and 1C:2G, each at a base concentration of 0.74 mM. The equimolar mixture shows greater hypochromicity than the 1C:2G mixture. The melting of this G + C complex occurs over a 15-20° range, which is much broader than is seen with other G + C complexes, and there is no evidence of more than one phase. If it is assumed that the first reading, at 6°, represents the true aggregated base-line, the midpoint of this transition is in the neighborhood of 15°. The difference spectrum corresponds with that for poly C + GpG (Fig. 7).

Effects of 2',5'-Linkage—The G + C complex resulting from the interaction of equal amounts of poly C and 2',5'-GpG, at a concentration of 0.6 mM base, melts at 19.4°. The complex formed with the normal 3',5'-GpG under the same concentration of 0.6 mM base, melts at 19.4°. The complex presents the true aggregated base-line, the midpoint of this transition is in the neighborhood of 15°. The difference spectrum corresponds with that for poly C + GpG (Fig. 7).

The stabilizing effect of the G-C bond in DNA that was noted by Marmur and Doty (10) is also reflected here in the relative strengths of bonding at the oligonucleotide level. Thus, under conditions in which (poly C + GpG) melts at 26.3°, it was found (14) that (polyribouridylic acid + ApA) melted at 6.2°. Hydrogen-bonded structures involving guanine seem to be extraordinarily stable, whether they be of the G + G or the G + C variety.

The relatively lower stabilities of the G-C complexes formed with GpGpUp and 2',5'-GpG, as compared with 3',5'-GpG, are consistent with the corresponding experiments in the polyribouridylic acid-adenine oligonucleotide series. Normally linked all-adenine oligonucleotides were shown to form stable complexes with polyribouridylic acid than did adenine oligonucleotides containing one unbonding uridine moiety at the end or containing 2',5'-linkages in place of the usual 3',5'-bonding.

The structures of the G + G + C and C + G + C complexes are undetermined. The melting of the second strand of G from the G + G + C complex is accompanied by the time dependence and the difference spectrum associated with the breaking of a G-G bond. If we assume a Watson-Crick type bonding for the G + C core, the G + G + C complex may well look like Fig. 8A. Such a structure is sterically feasible with models. A similar position for the second strand of poly C in the C + G + C complex is also possible, provided that the second strand is protonated to allow the bond to the carbonyl group on the guanine (Fig. 8B). This structure is consistent with the finding that the C + G + C complex is observed only in the pH region where poly C is partially protonated.

Summary

1. Polyribotydylate acid reacts quickly with members of the GpG . . . Gp and GpGp . . . G series to form two-stranded 1:1 complexes and three-stranded 2C:1G complexes. The nature of the complex formed is governed by the extent of protonation of the polyribotydylate acid under the conditions of the interaction.

2. The 1:1 GC complex reacts slowly with another mole of polyguanylic acid to form a three-stranded complex containing 1C:2G.

3. For a given guanine oligonucleotide, the dissociation temperatures of the G + C and C + G + C complexes are approximately the same. The dissociation of the G + G + C complex is biphasic, and the second phase of this melting occurs at the temperature of the dissociation of GC.

4. Spectral changes accompanying the formation of the GC bond are characterized in general by a difference spectrum showing maxima at about 240 to 245 mm and 270 mm and a minimum at 260 mm. Above 290 mm, the aggregation spectrum is hyperchromic to that of the dissociated mixture.

5. Interaction of polyribotydylate acid with GpGpUp and with 2',5'-GpG has also been demonstrated. These complexes are less stable than those formed with the corresponding normally linked all-guanine oligonucleotides.

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References

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