THE INTERACTIONS OF POLY C AND GUANINE TRINUCLEOTIDE

Marie N. Lipsett

National Institutes of Health, Bethesda, Maryland

Received April 1, 1963

The formation of hydrogen-bonded complexes between polyribocytidylic and polyriboguanylic acids has not yet been described, although many studies have been carried out on the corresponding A-U interactions (1). Adenine oligonucleotides have been shown to form helical structures with polyuridylic acid (2) or, in the deoxy series, with thymine oligonucleotides (3). Poly G-poly C studies are complicated by the strong self-bonding which poly G itself can undergo (4); since the self-bonding of GpGpGp is much weaker, the combination of poly C with this oligonucleotide is discussed here. The bonding with other guanine oligonucleotides such as GpG, GpGp, GpGpG, etc., has also been studied [1/].

A conventional mixing curve (1) for poly C and GpGpGp, when incubated at room temperature, shows a minimum at mole fraction 0.50 after four days (Fig. 1A), although readings taken before complete equilibration may show less sharp minima displaced more toward the C-rich end of the curve [2/]. Incubation

---

[1/] These oligonucleotides were prepared either by alkaline degradation of poly G or by polymerization of guanosine-2',3'-cyclic phosphate with takadiastase T_1 enzyme (5). Mixtures were separated on DEAE-urea columns (6).

[2/] At some wavelengths there is a downward curvature of the G-rich wing of the mixing curve which is a result of the strongly concentration-dependent interaction of the excess G-residues with each other, as has been described in the deoxy-G series (7). Since the self-aggregation of guanine oligonucleotides exhibits practically no hypochromicity at 225 or 290 mμ (Fig. 2), where the G+G+C structure shows changes in molar extinction of -0.70×10^3 and -0.41×10^3, respectively, these wavelengths were used to follow interactions.

of the same solutions at 3°C for two weeks results in a shift of the minimum to mole fraction G=0.66 (Fig. 1B), indicating the more stable complex is now G+G+C [3].

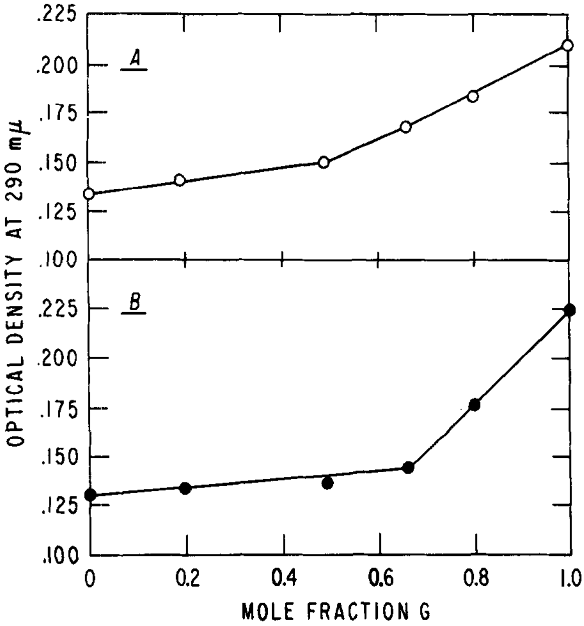

Fig. 1. Mixing curves of poly C and GpGpGp. Conditions: 0.2 M NaCl, 2 x 10^{-3} M cacodylate buffer, pH 6.2, 2 x 10^{-4} M nucleotide phosphate. A. Incubated at room temperature 4 days. B. Incubated at 3°C 2 weeks, read below 10°C.

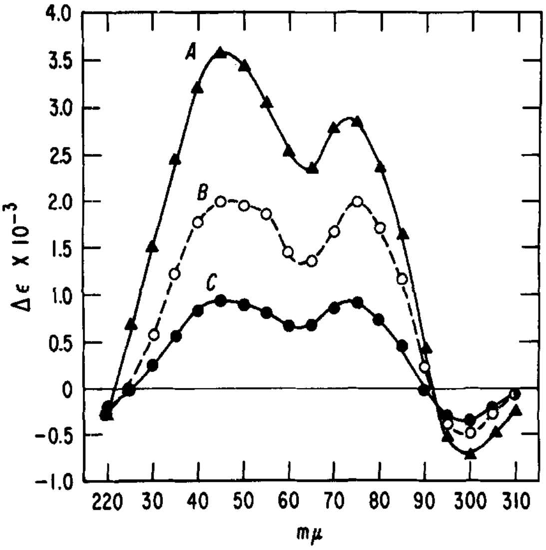

Fig. 2. Difference spectra of poly C-GpGpGp interactions. Solvent as in Fig. 1. A. Over-all change in molar extinction of G+G+C complex on melting, 10-53°C. B. Change in molar extinction on melting of GpGpGp alone. C. Change in molar extinction of G+G+C complex, G= 0.66, during first phase of melting, 10-32°C.

---

[3] J. Fresco has informed us that this structure is also produced in poly C--poly G interaction.

The specific rotations of these complexes are given in Table I. Because in concentrated solution at this pH both poly C and GpGpGp form highly dextrorotatory structures [4/] which in turn are disrupted by increasing temperature, it is not possible to obtain a mixing curve using rotation as a parameter. The $[\alpha]_{365}$ of +930° found for G+G+C, however, is far higher than the rotation which can be calculated assuming that the species present are C+G and self-bonded G+G, namely, +610°. At 66°C, where the structures are melted out, the discrepancy between the values found and calculated is only 6°. These data, taken together with the mixing curves, provide evidence for the existence at low temperatures of a G+G+C complex distinctly different from G+C or G+G.

Table 1. Specific Rotations [a/]

	$[\alpha]_D$			$[\alpha]_{365}$		
	10°C	32°	66°	10°	32°	66°
Poly C	+370	+380	+242	+1890	+1600	+964
GpGpGp	+108	-81	--	+430	-60	-47
Poly C + GpGpGp 1:1		+110	+40		+700	+439
Poly C + GpGpGp 1:2	+113	+86	+57	+930	+586	+283

[a/] Same conditions as in Fig. 1. Values are accurate to within 20°.

[4/] The sign of the rotation of the GpGpGp structure indicates that this structure differs from that of 5'-GMP (8), which exhibits a strong levorotation. The exact magnitude of rotation of GpGpGp is not known, for there is no indication that the solutions measured here were either completely or perfectly aggregated. However, the $[\alpha]_D$ of +108° for the aggregated form is comparable to the value of +100° reported for a poly G helix (4).

The melting curve of the G+G+C structure is biphasic (Fig. 3), in contrast to the monophasic melting of the A+U+U complex at this ionic strength. The first phase, up to 32°C, is extremely time-dependent, a characteristic of the G-G bond (9). As much as 8 hours may be required for equilibration near the inflection temperature of the curve. The T_m of this first phase of the curve, 24.7°, may be compared with the T_m of 23.7° found when a solution containing the same concentration of GpGpGp without the poly C is melted out the same time. The exact configuration of this all-G aggregate has not been established, but its stability is enough lower than that of the corresponding bonds in G+G+C to allow the latter to be formed preferentially. In addition the rapid rate of formation of the G+C complex at 3°C and the steep concentration-dependence of the G+G aggregate would probably favor the G+G+C structure even if the G-bondings in G+G and in G+G+C were equally stable.

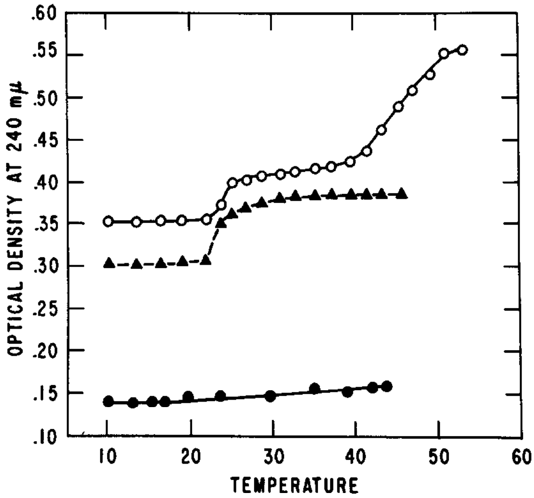

Fig. 3. Melting of poly C and GpGpGp aggregates. Solvent as in Fig. 1. 0-0-0 Poly C, 3×10^{-4} M base; X-X-X GpGpGp, 4.2×10^{-4} M base; ●-●-● Poly C + GpGpGp, mole fraction G=0.66, 6.3×10^{-4} M total base.

The second phase of melting is rapidly reversible, and optical equilibrium is reached as early as thermal equilibrium. This phase, which here melts above 32°, represents the breakdown of the G+C complex which was formed at room temperature, as seen in Fig. 1A.

Thus, it appears that the helical C+G structure can bond a second strand of G. This is a reversible step, and the second strand of G may be melted off without disrupting the C+G core of the structure.

REFERENCES

1. Warner, R.C., J. Biol. Chem. 229, 711 (1957); Felsenfeld, G., Davies, D.R., and Rich, A., J. Am. Chem. Soc. 79, 2023 (1957).
2. Lipsett, M.N., Heppel, L.A., and Bradley, D.F., J. Biol. Chem. 236, 857 (1961).
3. Gilham, P.T., J. Am. Chem. Soc. 84, 1311 (1962).
4. Fresco, J.R., and Massoulié, J., J. Am. Chem. Soc. in press.
5. Sato-Asano, K., and Egami, F., Biochim. Biophys. Acta 29, 655 (1958).
6. Lipsett, M.N., and Heppel, L.A., J. Am. Chem. Soc. 85, 118 (1963).
7. Ralph, R.K., Connors, W.J., and Khorana, H.G., J. Am. Chem. Soc. 84, 2265 (1962).
8. Gellert, M., Lipsett, M.N., and Davies, D.R., Proc. Nat. Acad. Sci. (U.S.) 48, 2013 (1962).
9. Khorana, H.G., personal communication.