INFRARED DEMONSTRATION OF TWO- AND THREE-STRAND HELIX FORMATION BETWEEN POLY C AND GUANOSINE MONONUCLEOTIDES AND OLIGONUCLEOTIDES

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The reaction of complementary homopolynucleotides to form helical secondary structures has been extensively studied and reviewed (1) and the formation of analogous structures between high polymers and oligonucleotides has been reported in several studies (2,3). We report here infrared spectral evidence for the formation of an ordered secondary structure between poly C[2/] and a mononucleotide, either 5'-GMP or 5'-dGMP[3/].

The analogous reactions of the guanosine oligonucleotides with poly C, for which the stoichiometry has been established (3), support the interpretation of the monomer-polymer spectra and permit their application to more highly polymerized systems. With 5'-GMP and with guanosine oligonucleotides it is possible to study G-C interactions

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1/ This investigation was supported by Public Health Service Post-doctoral Fellowship No. 2-F2-CA16,237-02 from the National Cancer Institute.

2/ Abbreviations used: 5'-GMP, guanosine-5'-phosphate; 5'-dGMP, deoxyguanosine-5'-phosphate; poly C, polycytidylic acid; GpGpGp, guanylyl-(3'-5')-guanylyl-(3'-5')-guanosine-3'-phosphate; G, guanine; C, cytosine; GpG, guanylyl-(3'-5')-guanosine.

3/ The same reactions are observed for both 5'-GMP and 5'-dGMP. The advantage of the latter is that it has a much weaker self-interaction.

under conditions where strong interactions between guanines can be demonstrated not to interfere with and obscure their reactions with poly C (3).

A solution of 5'-dGMP and poly C in a 1:1 ratio at 6° and pD 7.8 has the infrared spectrum shown in Fig. 1A[4/]. This spectrum changes with temperature in the manner shown in Figs. 1 and 2, finally

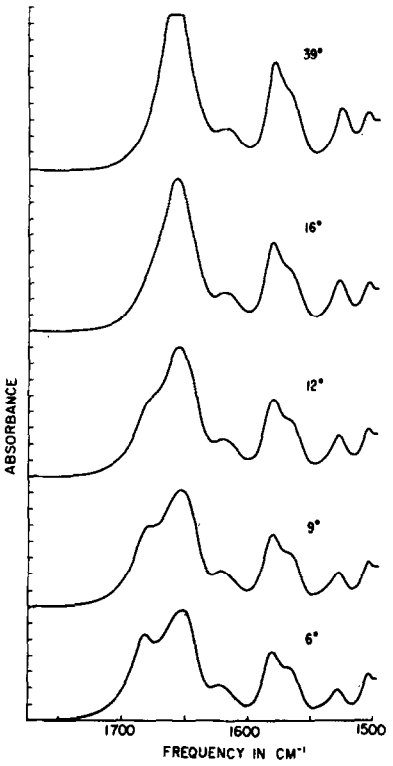

Fig. 1. Infrared spectra in D_2O solution of 5'-dGMP (0.115 M) plus poly C (0.115 M), 0.2 M phosphate buffer, pD 7.8; 55.6 μ path length. The mixture was chilled for several hours at 1° and the spectra measured as the temperature increased. The 6° spectrum is the one referred to in the text as curve "1A". Index marks on the ordinate are 0.1 absorbancy units apart in this as well as all succeeding figures.

4/ Methods and materials have been described in recent publications, (3,4).

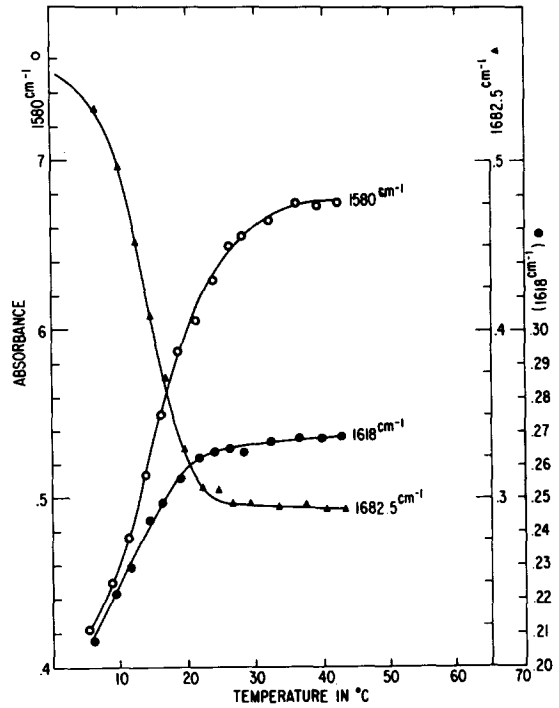

Fig. 2. Melting curves for 5'-dGMP plus poly C (1:1) interaction product under conditions given in Fig. 1. The lack of a plateau at the lower temperatures may be due to incomplete interaction.

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reaching at ~ 40° a spectrum identical with a summation spectrum of its components. The parallel melting behavior of bands which clearly originate from guanine and cytosine vibrations respectively (Table I), indicates that the spectrum in Fig. 1A represents an interaction between the two species rather than concurrent formation of two self-structures. A 1:1 stoichiometry for this interaction is inferred from the similarity of Fig. 1A to the spectrum of poly C + GpGpGp, Fig. 5, obtained under conditions which were recently shown to lead to interaction in this ratio (3). We interpret these results to indicate a specific cooperative interaction between the components, and suggest that the spectral changes result from a Watson-Crick pairing between the bases in a helical configuration.

The assignment of the guanylic acid carbonyl group to 1682 cm^{-1}

TABLE I

Absorption Maxima in cm^{-1}

Material[1]	Figure[2]	Secondary Structure	Band Assignments: Protonated cytosine ring vibration	Guanine carbonyl vibration	Cytosine carbonyl vibration[3]	Unprotonated cytosine ring vibration[5]	Guanine ring vibration
5'-dGMP + poly C	1A	two-stranded helix	-	1682	1652	1622	1581 1568
5'-dGMP + 2poly C	3A	three-stranded helix	1707	1687	1656[4]	1623	1582 1565
GpGpGp + poly C	5	two-stranded helix	-	1679	1650	1621	1581 1561
GpG + 2 poly C	6	three-stranded helix	1707	1684	1655[4]	-	1580 1568

[1] Infrared spectra and band assignments of guanosine, the guanylic acids and of poly C have been reported previously[6,7,8].

[2] Experimental conditions given in legends to figures.

[3] To the extent that interaction is incomplete there would be a contribution from uninteracted guanine carbonyl vibrations on the high frequency side of this band.

[4] We assign these bands to predominantly carbonyl vibrations in both the protonated and unprotonated cytosines.

[5] The bands at 1528 and 1503 in Figs. 1A and 3A are attributable to cytosine but are not assigned more specifically at present.

(Table I) is based upon observation of the interaction of 6-O^{18}-labelled 5'-GMP (5) with poly C. This isotopic substitution results in a decrease of the absorption maximum at 1682 cm^{-1} to 1674 cm^{-1}, indicating that this is a guanine and not a cytosine band. We assign the 1652 cm^{-1} band primarily to a cytosine carbonyl vibration because cytosine has a band near this frequency[5], guanine has no band near this frequency, and the guanine carbonyl band is already accounted for at 1682 cm^{-1}.

At low temperatures, a new interaction product was detected by the appearance of another band at 1707 cm^{-1} when the pD of a poly C + 5'-dGMP solution was reduced appreciably below 8. The 1707 cm^{-1} band first appeared at ~ pD 7.6[6], reached a maximum value at pD ~ 6.4 ($\epsilon \cong 300$)[7] and remained constant in intensity down to pD ~ 4.6. A typical spectrum observed in this pD range at a C:G ratio of 2:1 is shown in Fig. 3A. The increase of intensity of the 1707 cm^{-1} band observed by changing the C:G ratio from 1:1 to 2:1 at an acid pD, or by decreasing the pD below 8 at a C:G ratio of 2:1, led us to assign this band to a protonated cytosine ring vibration rather than to a

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[5] The spectra of cytidine, acid cytidine and poly C (6) and of guanosine, guanylic acids and guanylic acid gels (7,8) have been published.

[6] The high pD which is necessary to avoid protonation of C indicates that the formation of this double-stranded structure is accompanied by an unusually large change (between 3 and 4 pK units) in effective pK of the cytosine residues. Most of the pK shifts resulting from secondary structure of nucleic acids are between 1 and 2 pK units, cf., for example (9).

[7] The spectra are readily reproducible on a qualitative basis though there is some quantitative variation in peak intensities. For example, in four observations of different solutions of 5'-GMP and poly C (1:2 ratio) the ϵ_{1707} values had an average value of 300 (conc. based on total nucleotide) with an average deviation of 2%, but in two other determinations the values were about 15% lower than the average. In general, the experimental difficulties encountered with this system lead to a somewhat lower precision than we have obtained with mixtures of homopolymers. Even when the absolute values have differed, however, the frequencies, band shapes, and relative peak intensities were essentially the same.

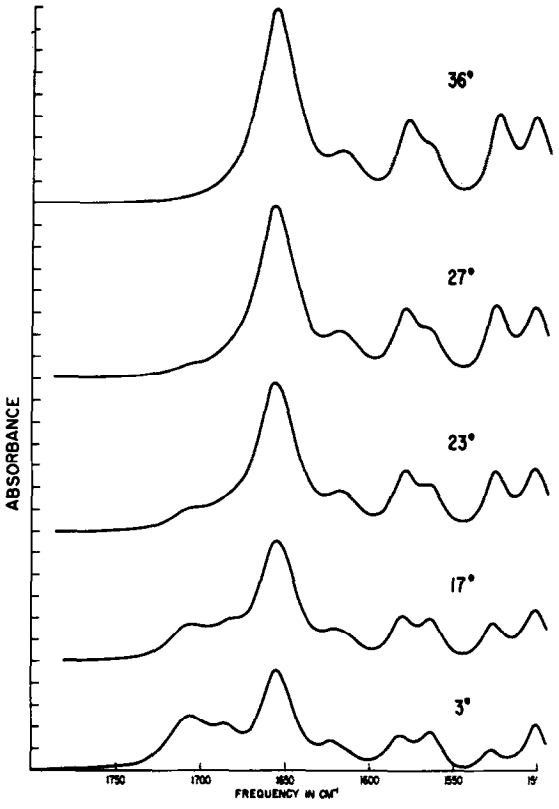

Fig. 3. Infrared spectra in D_2O solution of 5'-dGMP (0.054 M) plus poly C (0.109 M); 0.2 M sodium cacodylate, pD 7.0; 55.6 μ path length. The solution was chilled for several hours at 1°. Spectra were measured as the temperature increased. The 3° spectrum is the one referred to in the text as curve "3A".

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shifted guanosine carbonyl vibration. Confirmation of this assignment was obtained by observing the interaction product of poly C with 5'-GMP labelled in the carbonyl position with O^{18} (5). This spectrum showed no change in frequency of the 1707 cm^{-1} band although there was a decrease of the absorption maximum at 1687 cm^{-1} to 1680 cm^{-1}. We infer the absence of poly C and of guanylic acid self-structures (10,11) from the lack of bands at 1595 cm^{-1} and 1608 cm^{-1}, respec-

tively[8]. Finally, as before, the fact that the spectrum in Fig. 3A reflects a specific interaction between G and C rather than concurrent formation of two self-structures can be demonstrated by the parallel melting behavior of bands clearly assignable to the two different bases (Fig. 4).

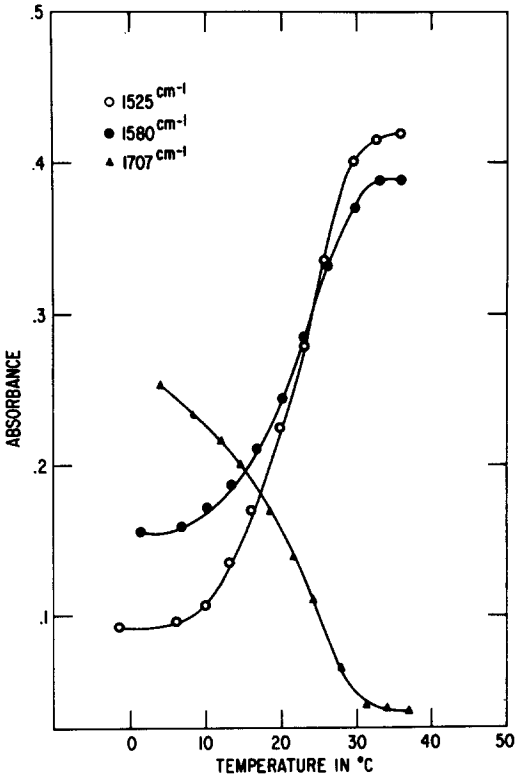

Fig. 4. Melting curves for 5'-dGMP plus poly C (1:2) interaction product under conditions given in Fig. 3.

These spectra indicate the presence of both neutral poly C and protonated poly C in the complex with 5'-dGMP. If one assumes that the structure must be based upon a reasonable hydrogen bonding scheme

8/ A band at 1595 cm^{-1} is characteristic of gel formation with both 5'-GMP and 5'-GMP-6-O^{18} (7,8). We have carried out infrared studies (Frazier, J., and Miles, H. T., to be published) of the helical molecule formed by half-protonated poly C and found the principal bands in the double bond region at 1694 cm^{-1} (protonated ring vibration), 1664 cm^{-1} (carbonyl vibrations of both protonated and unprotonated cytosines) and 1608 cm^{-1} (ring vibrations of the half of the cytosines which are not protonated).

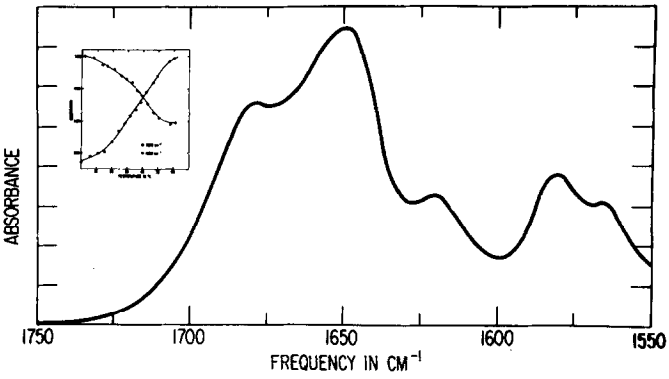

Fig. 5. Infrared spectrum in D_2O solution of GpGpGp + 2 poly C, 0.13 M sodium cacodylate, pD 8.3; 23.8 μ path length; fivefold ordinate expansion; temperature 5-6° C. This solution had been stored for 15 days at 5° C, but cooling periods of a few hours resulted in closely similar spectra. Melting curves at 1680 cm^{-1} and 1580 cm^{-1} are shown. All spectral changes on heating and cooling were reversible. The excess of poly C resulted in more intense cytosine carbonyl and ring vibrations, but did not change the more characteristic guanosine vibrations or the general similarity of this spectrum to that shown in Fig. 1A.

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and that the components combine in integral ratios, the most reasonable interpretation is that the stoichiometry is represented by C_2G and that the structure is three-stranded. Further support of this interpretation is obtained by observing that the infrared spectrum of GpG + 2 poly C (Fig. 6), for which the stoichiometry has been demonstrated (3), is essentially the same as that shown in Fig. 3A.

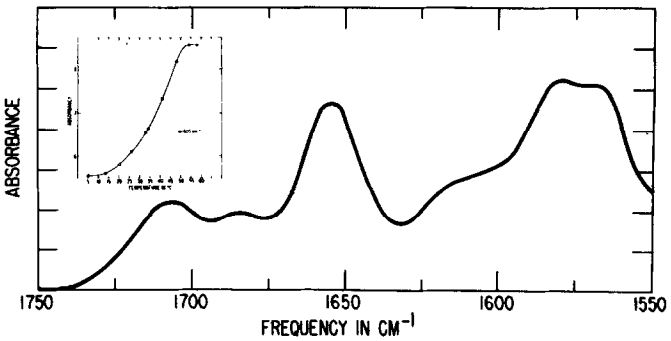

Fig. 6. Infrared spectrum in D_2O solution of GpG + 2 poly C, concentration 0.036 M (based on total ribose present); 0.06 M sodium cacodylate, pD 6.7; 55.7 μ path length; fourfold ordinate scale expansion; temperature 5-6°. The solution had been stored at 5° for 24 hours before measurement. The melting curve at 1655 cm^{-1} is shown. The bands below ~ 1625 cm^{-1} are obscured by a contaminant, presumably from a column used in the purification of GpG (3).

It is probable that 5'-GMP forms bonds with one strand of neutral poly C in the Watson-Crick manner, and with a strand of protonated poly C using the $C_6 = O$ and N_7 positions. There are two plausible bonding schemes for this structure, represented by I and II (Fig. 7).

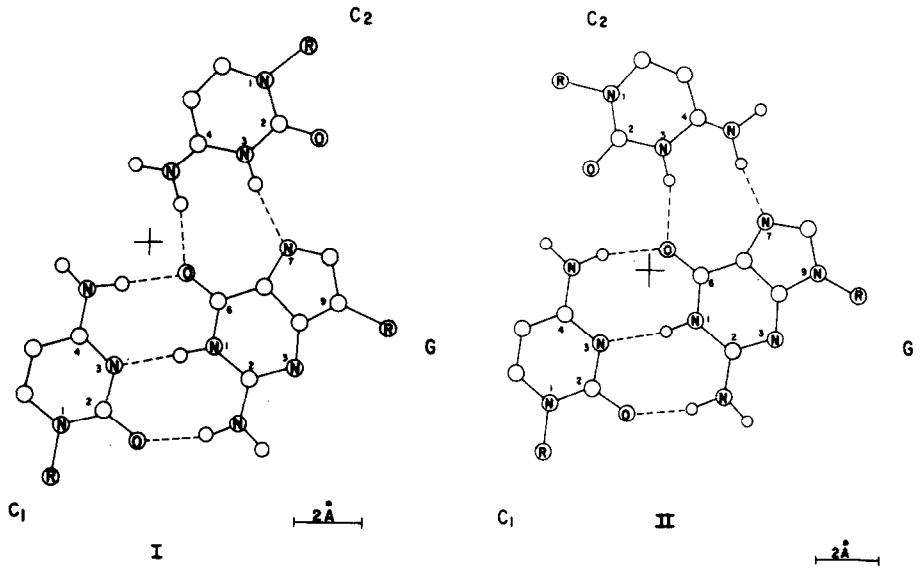

Fig. 7. Possible bonding schemes between G and C in three strand helix formed between 5'-GMP and 2 poly C.

The Watson-Crick strands are antiparallel in both, but in I the two poly C chains have an antiparallel, and in II a parallel polarity. We have built three-dimensional models based on both bonding schemes (cf. 12). Structure I appears to have a larger diameter and places the ribose-phosphate chain of the C_2 strand in closer proximity to the phosphates of the 5'-GMP residues than is the case with II. Structure II, as we have built it, has some short contacts between the 2'-hydroxyl oxygens of the C_2 strand and the next adjacent cytosine residues in the same chain. We favor structure II which has previously been suggested for the analogous oligonucleotide-poly C structure reported in (3).

The present communication appears to be the first report of a formation of helical interaction products between a mononucleotide and

a polynucleotide. More detailed studies of monomer-polymer interactions are in progress and will be reported at a later date.

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