BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 3391—3399 (1970)

Structure of Polynucleotide Complex with Non-Complementary Nucleosides. I. Poly A,G+Poly U

Hideo AKUTSU and Masamichi TSUBOI

Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo

(Received June 4, 1970)

Copolymers of riboadenylic and riboguanylic acids (poly A,G) with guanylic acid contents of $8._5$—$29._6$% were prepared. The ultraviolet and infrared absorptions were examined of aqueous solutions of mixtures of poly A,G and polyribouridylic acid (poly U) of various mole ratios. It has been shown that in a solvent with 0.1 M Na^+ a triple-helical structure is formed with a A$\begin{matrix}\cdot\cdot U\\ \cdot\cdot U\end{matrix}$ type inter-base binding as well as a double-helical structure with a A···U type binding, while in a solvent with 0.01 M Na^+ only double-helical structure is formed. A guanine residue in the copolymer remains within the helix structure when its neighbors are mostly adenine residues, whereas it rotates out when there are many guanine residues in its vicity. On heating the solution, the structures just mentioned were found to be broken. The process of such a breaking down was followed also by means of the ultraviolet and infrared absorption measurements.

Polyriboadenylic acid (poly A) and polyribouridylic acid (poly U) are known to form a double- or triple-stranded helical structure in aqueous solution (see Figs. 1 (a) and 1(b)).[1,2] In such a structure, adenosine (A) and uridine (U) residues are considered to be strongly bound with each other through specific hydrogen bonds; A and U are considered to be complementary. In the present work, we have introduced the guanosine residue (G), which is not complementary to U, into the poly A chain, to examine its effect upon the structure. The question is whether the structure becomes like Fig. 1(c), 1(d), 1(e), or 1(f). In the structure Fig. 1(c) or 1(d), the G residues remain within the interior of the helix. They merely replace the A residues, and the outline of the structure here remains almost the same with that without the G residues (Fig. 1 (a) or 1 (b)). While, in the structure Fig. 1 (e) or 1 (f), the G residues rotate out of the helix in such a manner as to enable complementary A···U binding in the the subsequent regions of the polynucleotide chains.

As was shown previously,[3-8] the structures (c), (d), (e), and (f) in Fig. 1 can be distinguished by examining the mixing curve—*i.e.*, by examining the mole ratio poly A, G/poly U (on the basis of the phosphorus analysis), at which the maximum hypochromicity (minimum absorbance) appears for a proper ultraviolet radiation (at 259 mμ, for example). The mole ratio should be 50/50 for (c), 33.3/66.7 for (d), $100/x$ for (e), and $100/2x$ for (f), where x(%) is the mole % of A in the copolymer of A and G (poly A, G) now in question. On the other hand, the hydrogen-bonded A···U and A$\begin{matrix}\cdot\cdot U\\ \cdot\cdot U\end{matrix}$ structures are known to give characteristic infrared absorptions in D_2O solution which are distinguished from each other and from A and U residues free from hydrogen-bonds.[9] We attempted the ultraviolet and infrared absorption measurements of our poly A, G+poly U in aqueous solutions, keeping these previous demonstrations in mind.

Preparation of the Polynucleotide Samples

The polynucleotide samples used were prepared by the use of polynucleotide phosphorylase obtained from *Micrococcus lysodeikticus* or from *Azotobacter vinelandii*. The compositions of the reaction mixtures and the incubation times are given

1) A. Rich and D. R. Davies, *J. Amer. Chem. Soc.*, **78**, 3548 (1956).
2) G. Felsenfeld, D. R. Davies and A. Rich, *ibid.*, **79**, 2023 (1957).
3) J. R. Fresco and B. M. Alberts, *Proc. Nat. Acad. Sci. U. S.*, **46**, 311 (1960).
4) R. F. Steiner, *J. Biol. Chem.*, **235**, 2946 (1960); **236**, 342 (1961).
5) W. Szer and D. Shugar, *Acta Biochim. Pol.*, **8**, 235 (1961).
6) M. N. Lipset, L. A. Heppel and D. F. Bradley, *J. Biol. Chem.*, **236**, 857 (1961).
7) T. Ichikawa, H. Fujita, K. Matsuo and M. Tsuboi, This Bulletin, **40**, 2272 (1967).
8) M. Tsuboi, K. Matsuo, and M. Nakanishi, *Biopolymers*, **6**, 123 (1968).
9) H. T. Miles and J. Frazier, *Biochem. Biophys. Res. Commun.*, **14**, 21 (1964).

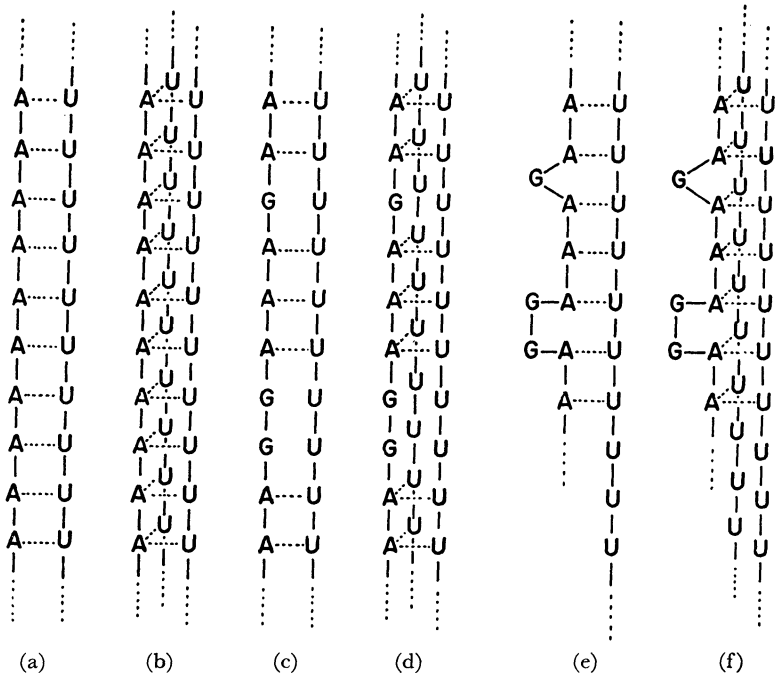

Fig. 1. Schematic drawings of possible structures of polynucleotide complexes.
(a) Complete double-helix.
(b) Complete triple helix.
(c) Double-helix with the G residues inside.
(d) Triple-helix with the G residues inside.
(e) Double-helix with the G residues looping out.
(f) Triple-helix with the G residues looping out.

Table 1. An example of reaction mixture in enzymatic preparation of polynucleotide

Buffer solution[a)]	1.0 m*l*
Enzyme solution	1.5[b)]
Adenosine-5′-diphosphate (21 mg/m*l*)	1.1
Adenosine-5′-triphosphate (25 mg/m*l*)	0.5
Guanosine-5′-diphosphate (11 mg/m*l*)	0.9
$(NH_4)_2SO_4$ solution (saturated)	1.25
Total	6.25 m*l*

a) This buffer solution was prepared by mixing 1.25 m*l* of 2 M Tris, 3 g of urea, and 2 m*l* of 2 M $MgSO_4$, by adding 12 M HCl to reach pH 9, and then by adding 0.5 M mercaptoethanol so that the total volume become 25 m*l*.
b) Usually this amount contains 10—20 units of enzyme. 1 units=amount of enzyme which can liberate 1 μmol of orthophosphate in the enzymatic reaction of 15 min. The most efficient amount of enzyme was determined by several trial experiments with smaller scale (1/200 of that described here).

in Tables 1 and 2. The incubation temperature was 37°C for the enzyme from *M. lysodeikticus* and 30°C for that from *A. vinelandii*. After the enzymatic reaction, the product was precipitated with perchloric acid or with ethanol and then purified by the method of phenol extraction and by dialysis.

Base Composition and Some Other Characterization of the Copolymers

The base composition of each poly A,G sample was determined by hydrolyzing it with 1 N HCl, by the paper-chromatographic separation of the resulting adenine and guanine, and by ultraviolet absorption measurements of them. The composition thus determined is given in Table 2. The base sequences in these copolymers are not known, but it is probable that they are nearly random. Saigo, Uchida and Egami (private communication) made an analysis of the products obtained by a digest of a poly A,G in which G content is 30% with ribonuclease T_1. In a DEAE-Sephadex column chromatography with 7M urea they found more than ten peaks, each of which corresponds to $(A_p)_nG_p$ where $n=0,1,2,\cdots$. The relative amounts of these oligonucleotides were not found to be greatly different from what are expected for the random sequence.

The degrees of polymerization of the polymers prepared were not determined, but they are considered to be sufficiently high on the basis of the

Table 2. Enzymatic preparation of homopolymers of uridylic acid and copolymers of adenylic and guanylic acids

Polymer	Substrate[a] ADP mg	Substrate[a] GDP mg	Enzyme from	Incubation time hr	Product[d] Yield mg	A %	G %	Sedimentation coefficient S_{20}
Poly A,G(1)	18.0	2.0	*A.v.*	14.5	4.6	91.5	8.5	6.3
Poly A,G(2)	17.0	3.0	*A.v.*	14.5	6.5	85.5	14.5	3.7
Poly A,G(3)[b]	—	—	*M.l.*	—	—	80.0	20.0	7.7
Poly A,G(4)	23.2	9.9	*M.l.*	3.0	7.5	76.0	24.0	5.6
Poly A,G(5)	17.1	15.9	*M.l.*	3.0	3.9	70.4	29.6	6.2
Poly U	UDP 10 mg		*A.v.*	3.0	2.0	—	—	6.5
Poly U	UDP 40 mg		*A.v.*	3.0	11.7	—	—	5.2

a) ADP: adenosine-5′-diphosphate. GDP: guanosine-5′-diphosphate.
b) Prepared by Drs. T. Saigo, T. Uchida and F. Egami and kindly placed at our disposal by them.
c) *A.v.*: *Azotobacter vinelandii*. *M.l.*: *Micrococcus lysodeikticus*.
d) A: adenine residue content. G: guanine residue content.

sedimentation constants given in the last column of Table 2. These were measured in distilled water with no salt except for poly A,G(4) and poly A,G(5). The sedimentation constants of the latter two were examined in 0.1 M NaCl+0.01 M Na-cacodylate buffer (pH 6.5).

Experimental

Ultraviolet absorption measurements were made with an Ito spectrophotometer Model QU-3. The temperature of the samples was controlled as previously described.[10] The polynucleotide concentration was determined by measuring phosphorus content.[11]

Infrared absorption measurements were made of D_2O solutions by the use of a Perkin-Elmer 621 spectrometer. Each D_2O solution was placed in a cell with CaF_2 windows and of 50 μ in optical path length. The temperature of the solution was controlled by an electric heating device constructed by ourselves, and the temperature was measured by the use of a thermocouple.

UV Mixing Curves: Effect of Salt

Solutions of poly A,G and poly U of various mole ratios were made, and their ultraviolet absorbance at 259 mμ recorded. The results are shown in Figs. 2—6. From such an absorbance versus mole-ratio profile, we may judge whether the two polymers in question have no interaction or whether they form a complex. When a complex is formed, its stoichiometric ratio may also be determined from the observed profile of the mixing curve.

As may be seen in Figs. 2—6, every poly A,G shows evidence of interaction with poly U. Each of the mixing curves consists of two straight lines which intersect at a proper mole ratio. As has been stated in the introduction of this paper, the intersection should take place at poly A,G/poly U = 50/50, 33.3/66.7, 100/x, or 100/2x if the structure of the cmplex is respectively like (c), (d), (e), or (f) of Fig. 1. Actually, however, the intersection does not always take place exactly at one of these four expected mole ratios (see Figs. 2—6).

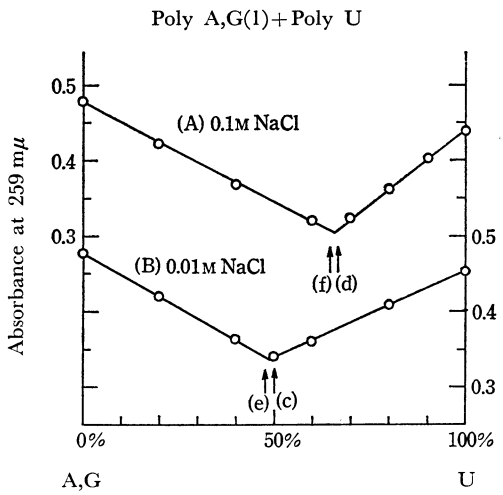

Fig. 2. Mixing curves for poly A,G(1) and poly U.
Abscissa: mol% (determined by measuring phosphorus contents) of poly U, ordinate: absorbance at 259 mμ. Total nucleotide concentration in the solution was kept at 0.48×10^{-4} M. (A) In 0.1 M NaCl plus 0.01 M Na-cacodylate buffer, pH 7.0; at 21.7°C. (B) In 0.01 M NaCl plus 0.001 M Tris buffer, pH 8.0; at 24.5°C. The vertical arrows indicate the positions of minimum expected for complexes with structures (c), (d), (e) and (f) given in Fig. 1.

10) K. Matsuo and M. Tsuboi, This Bulletin, **39**, 347 (1966).
11) B. N. Ames and D. T. Dubin, *J. Biol. Chem.*, **235**, 769 (1960).

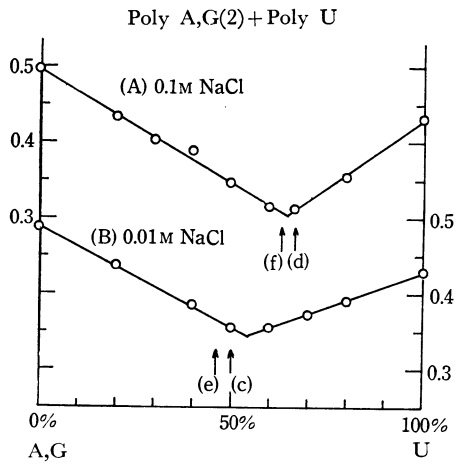

Fig. 3. Mixing curves for poly A,G(2) and poly U.
Abscissa: mol% of poly U, ordinate: absorbance at 259 mμ. Total nucleotide concentration in the solution was kept at 0.48×10^{-4} M. (A) In 0.1 M NaCl plus 0.01 Tris buffer, pH 8.0; at 23.6°C. (B) In 0.01 M NaCl plus 0.001 M Tris buffer, pH 8.0; at 23.6°C.

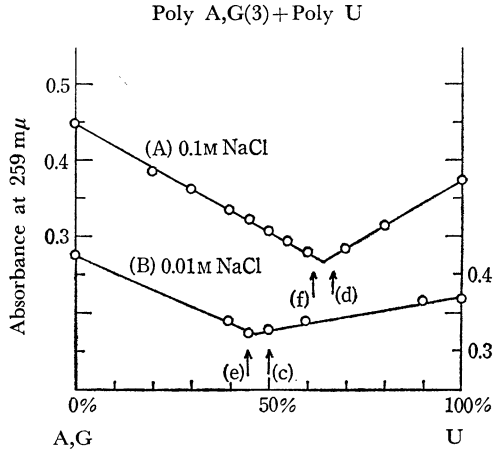

Fig. 4. Mixing curves for poly A,G(3) and poly U.
Abscissa: mol% of poly U, ordinate: absorbance at 259 mμ. Total nucleotide concentration in the solution was kept at 0.43×10^{-4} M. (A) In 0.1 M NaCl plus 0.01 M Na-cacodylate buffer, pH 6.5; at 9.8°C. (B) In 0.01 M NaCl plus 0.001 M Na-cacodylate buffer, pH 6.5; at 10.6°C.

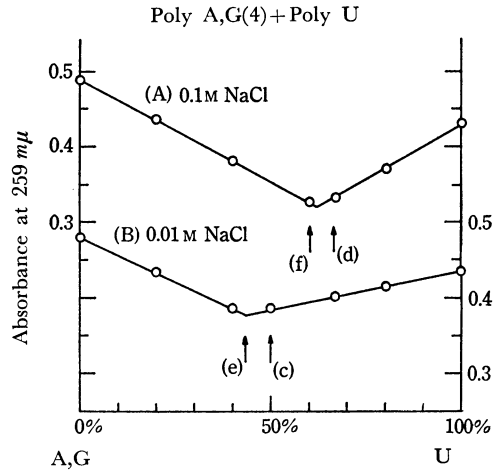

Fig. 5. Mixing curves for poly A,G(4) and poly U.
Abscissa: mol% of poly U, ordinate: absorbance at 259 mμ. Total nucleotide concentration in the solution was kept at 0.48×10^{-4} M. (A) In 0.1 M NaCl plus 0.01 M Tris buffer, pH 8.0; at 12.9°C. (B) In 0.01 M NaCl plus 0.001 M Na-cacodylate buffer, pH 7.0; at 12.6°C.

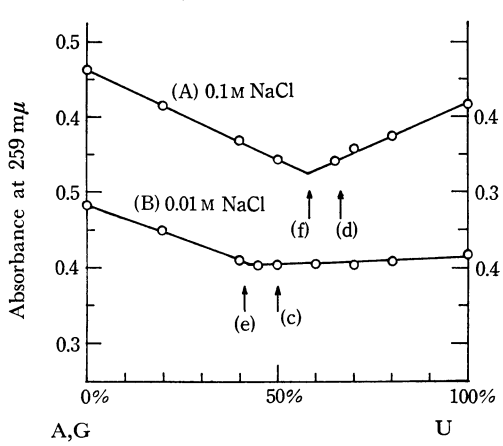

Fig. 6. Mixing curves for poly A,G(5) and poly U.
Abscissa: mol% of poly U, ordinate: absorbance at 259 mμ. Total nucleotide concentration in the solution was kept at 0.48×10^{-4} M. (A) In 0.1 M NaCl plus 0.01 M Na-cacodylate buffer, pH 7.0; at 13.0°C. (B) In 0.01 M NaCl plus 0.001 M Na-cacodylate buffer, pH 7.0; at 16.7°C.

This is in contrast to the case of poly A, U + poly U, where the stoichiometric ratio of the complex was found to be always $100/x$ or $100/2x$[3] (x= mole % of A in the copolymer of A and U).

The intersection appears always in the vicinity of poly A, G/poly U = 33.3/66.7 for solutions with 0.1 *M* NaCl, and in the vicinity of 50/50 for solutions with 0.01 *M* NaCl (with one exception). When the guanine content in poly A, G is smaller, the intersection appears at a point between those expected for (d) and (f) or between those expected for (c) and (e) (see Figs. 2, 3, and 4). When the guanine content in poly A, G is higher, on the other hand, the intersection appears at a point which is very near to that expected for (f) or (e) (see Figs. 5 and 6). These facts indicate that in a solvent with 0.1 *M* Na^+ a triple-helical structure is formed

Table 3. Observed and calculated minima in thf mixing curve of poly A,G and poly U

Copolymer	Total G %	G in A_pG_pA %	Minimum point in the mixing curve with poly U	
			Expected (% of U)	Observed (% of U)
Poly A,G(1)	8.5	7.4	66.4	66.0
Poly A,G(2)	14.5	10.8	65.7	65.0
Poly A,G(3)	20.0	12.8	65.0	64.5
Poly A,G(4)	24.0	13.9	64.2	62.5
Poly A,G(5)	29.6	14.7	62.9	58.0

with a A⋮U type inter-base hydrogen bonding, while in a solvent with 0.01 *M* Na^+ a double-helical structure is formed with a A···U type hydrogen-bonding. These facts also show that some of the guanine residues in the copolymer remain within the helix structure and some rotate out of the helix; in other words, some parts of the structure are like Fig. 1 (c) or (d) and some parts like (e) or (f). When the guanine content in the copolymer becomes greater the parts like (e) or (f) predominate. This may be taken as indicating that an isolated guanine residue is incorporated into the helix whereas a guanine cluster is looped out of the helix.

By assuming the random sequence, the amount of the isolated guanylic acid residue (that is the guanylic acid whose two neighbors are both adenylic acids, like $A_pG_pA_p$) was calculated from the total amount of the guanylic acid residue in each copolymer (Table 3). By assuming further that only such an isolated guanine residue is incorporated into the helix and that other guanine residues rotate out of the helix, the stoichiometric ratios poly A,G/poly U were calculated for poly A,G-poly U complexes. The results are given in Table 3. As may be seen here, the calculated ratio is always slightly higher than the observed ratio, and the discrepancy becomes greater as the guanylic acid content in the copolymer becomes higher. Thus, it is probable that a guanine residue is incorporated into the helix only when there are some adenine residues in its vicinity, and that the number of such adenine residues requires in its vicinity is *greater* than two.

Copolymer A,G(2) is found to be somewhat anomalous. Even in a solvent with 0.01 *M* Na^+ the minimum of the mixing curve (with poly U) does not appear in the vicinity of the mole ratio 50/50. Instead, the minimum is found with a good reproducibility at poly A, G/poly U = 45/55 (see Fig. 3). No explanation is yet given for this fact, but it may be pointed out here that, when a homopolymer poly A (instead of poly A,G) was mixed with poly U, the minimum of the mixing curve was always observed at poly A/poly U = 33.3/66.7, and never at 50/50.[12] Therefore, it is probable that copolymer A, G (2) happens to have a long···$A_pA_pA_p$···cluster in it, which behaves like a homopolymer poly A.

UV Mixing Curves: Effect of Temperature

The profile of the mixing curve just mentioned changes not only with the ionic strength but also with the temperature of the solution. As has been already described, the minimum in the mixing curve appears in the vicinity of poly A,G/poly U = 1/2 at a lower temperature in a higher ionic strength. As may be seen in Figs. 7 and 8, the minimum is shifted into the vicinity of poly A,G/poly U = 1/1 at an elevated temperature. This fact indicates that a triple-helical complex poly A,G–poly U–poly U first decomposes into double-

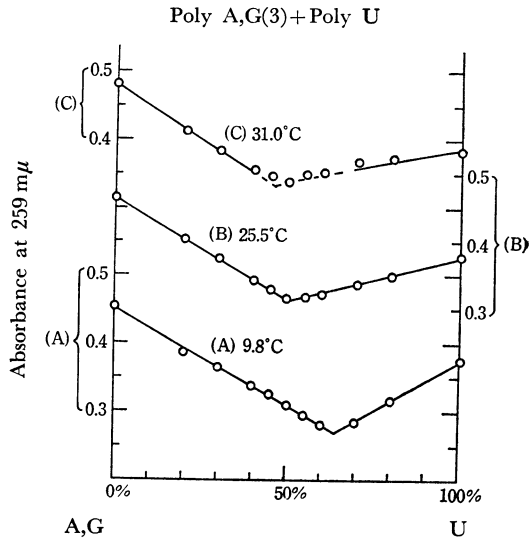

Fig. 7. Mixing curves for poly A,G(3) and poly U.
Abscissa: mol% of poly U, ordinate: absorbance at 259 mμ. Total nucleotide concentration in the solution was kept at 0.43×10^{-4} M. Solvent: 0.1 M NaCl plus 0.01 M Na-cacodylate buffer, pH 6.5. (A) At 9.8°C. (B) At 25.5°C. (C) At 31.0°C.

12) S. Higuchi and M. Tsuboi, This Bulletin, **39**, 1886 (1966).

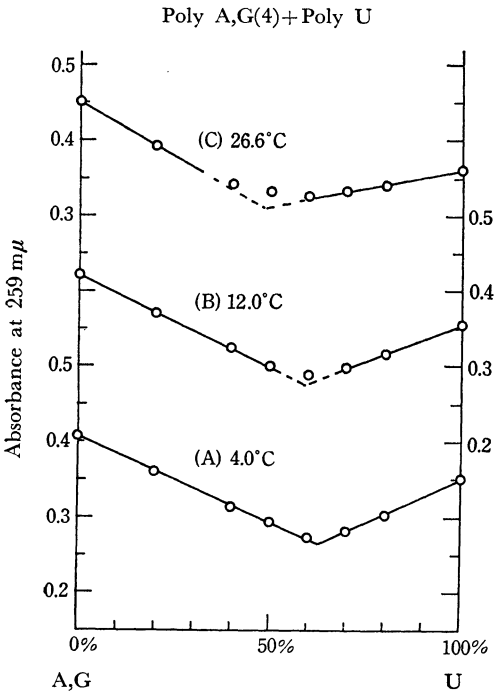

Fig. 8. Mixing curves for poly A,G(4) and poly U.
Abscissa: mol% of poly U, ordinate: absorbance at 259 mμ. Total nucleotide concentration in the solution was kept at 0.48×10^{-4} M. Solvent: 0.05 M NaCl plus 0.005 M Na-cacodylate buffer, pH 7.0. (A) At 4.0°C. (B) At 12.0°C. (C) At 26.6°C.

helical complex poly A,G–poly U and single-stranded poly U, before it completely melts into single-stranded random coils. Thus, the melting process is given as

$$\text{poly A,G}\cdot\text{poly U}\cdot\text{poly U} \longrightarrow \text{poly A,G}\cdot\text{poly U} + \text{poly U} \longrightarrow \text{poly A,G} + \text{poly U} + \text{poly U} \quad (1)$$

This is similar to the melting process known of the the triple-helical complex without G, *i.e.*, poly A–poly U–poly U.[12–14]

As is illustrated in Fig. 8, the minimum in a mixing curve observed at a higher temperature is often less sharp than that a at lower temperature. In the curve obtained at 12.0°C or at 26.6°C given in Fig. 8, the angle is rounded off at the minimum. For this, the last course of the melting process (1) is considered to be responsible. Thus, if a certain fraction of helices melts into random coils, a hyperchromicity takes place whose amount should be proportional to the amount of helices. The amount of hyperchromicity should be greatest at the minimum where the amount of helices is greatest. At an extremely high temperature, at which all the helices melt into random coils, the mixing curve should be a single straight line and have no minimum.

UV Heating Curves

Absorbance-temperature profiles of each of the 1 : 2 (molar) mixtures of poly A,G and poly U are shown in Figs. 9 and 10.

The triple-helical complex poly A,G(1)–poly U–poly U shows a two-step melting (at 43 and 51°C) in a solvent with 0.1 *M* Na^+ (Fig. 9(A)), whereas it shows a single-step sharp melting (at 34.5°C) in 0.01 *M* Na^+ (Fig. 10 (A)). Similar melting profiles were observed of the triple-helical complex without G, *i.e.* poly A–poly U–poly U.[12–14] For the latter, however, every melting temperature is found slightly higher than the corresponding melting temperature observed here for the complex with poly A,G(1). For the system of 1 : 2 (molar) mixture of poly A and poly U, a phase diagram shown in Fig. 11 was constructed.[12–14] For the system of 1 : 2 (molar) mixture of poly A,G(1) and poly U, now in question, a similar phase diagram would be valid. On the basis of the melting profiles just described (Figs. 9 (A) and 10 (A)), such a phase diagram is drawn with the broken lines in Fig. 11. The difference of the two phase diagrams (full and broken lines in Fig. 11) is considered to be caused by the 8.5% guanine residues in poly A,G(1).

As is shown in Figs. 9 and 10, the melting temperature becomes lower as the guanine content in

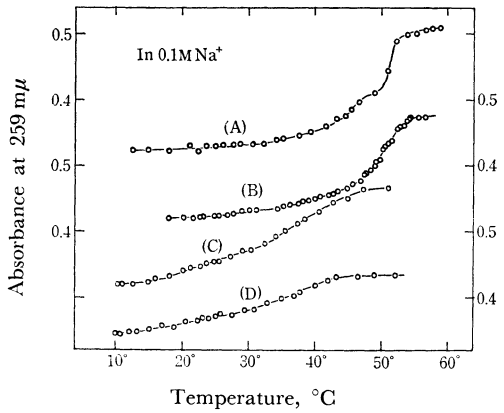

Fig. 9. Variation of the absorbance at 259 mμ with temperature of mixture solutions of poly A,G and poly U.
Mole ratio, poly A,G/poly U, was always 1/2. Solvent: 0.1 M NaCl plus buffer. (A) Poly A,G(1)+poly U. (B) Poly A,G(2)+poly U. (c) Poly A,G(4)+poly U. (D) Poly A,G(5)+poly U.

13) J. R. Fresco, In "Informational Macromolecules," ed. by H. J. Vogel, V. Bryson and J. Q. Lampen, Academic Press, New York (1963), p. 121.
14) C. L. Stevens and G. Felsenfeld, *Biopolymers*, **2**, 293 (1964).

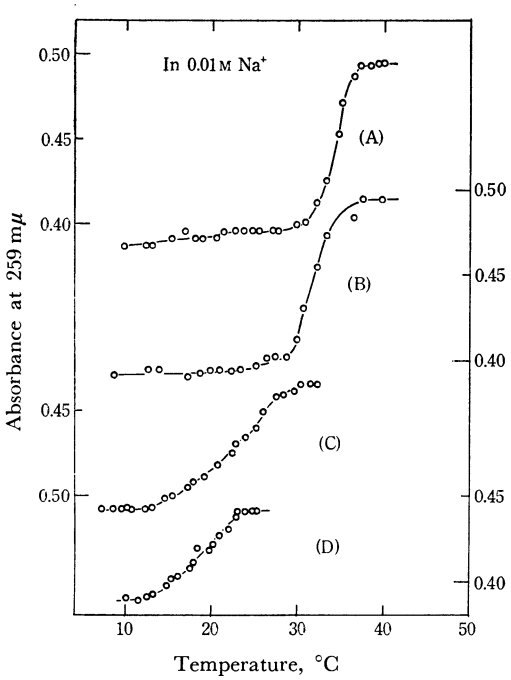

Fig. 10. Variation of the absorbance at 259 mμ with temperature of mixture solutions of poly A,G and poly U.
Mole ratio, poly A,G/poly U, was always 1/2. Solvent: 0.01 M NaCl plus buffer. (A) Poly A,G(1)+poly U. (B) Poly A,G(2)+poly U. (C) Poly A,G(4)+poly U. (D) Poly A,G(5)+poly U.

the copolymer poly A,G becomes higher. At the same time, the melting region becomes broader and total amount of hyperchromicity in the melting becomes smaller. These facts show that the secondary structure of a complex with poly A, G with a higher G content is less definite.

Infrared Spectra

Poly A,G(1) in heavy water solution (with 0.2 M Na^+) gives a strong absorption band at 1624 cm^{-1} at pD=7.0 and at 36°C (Fig. 12). This band is assignable to a double-bonds stretching vibration in the deuterated adenine ring.[15] On heating the solution this band is shifted slightly towards lower frequency (1620 cm^{-1} at 75°C).

Figure 13 shows the infrared spectrum of a 1 : 1 (molar) mixture of poly A,G(1) and poly U. At 38°C, it shows strong absorption peaks at 1689, 1671, and 1630 cm^{-1}. These are assigned respectively to $C_2=O$ stretching vibration of uracil, $C_4=O$ stretching vibration of uracil, and double-bonds stretching vibration of adenine residues involved in the Watson-Crick type A···U base pair.[15,16] This is definitely different from the infrared spectrum expected from AU type inter-base bonding plus free adenine residue.[15-17]

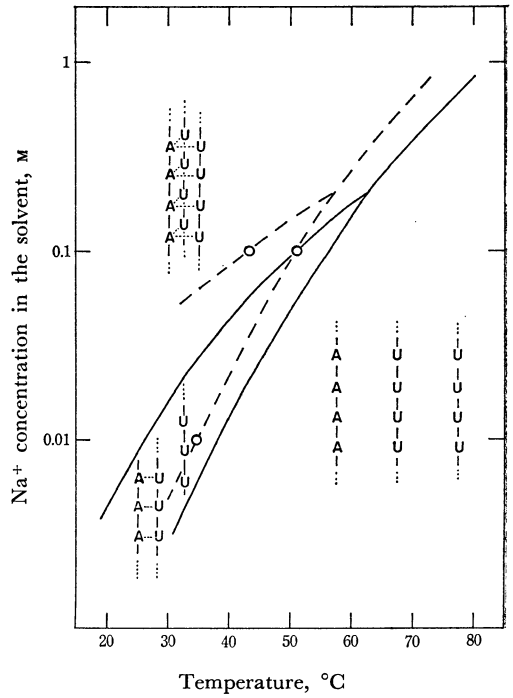

Fig. 11. Equilibrium diagram for 1:2 (molar) mixtures of poly A and poly U (full lines).
Broken lines show the effect of introduction of the guanine resideue into poly A. The small circles indicate melting temperatures observed for 1:2 complex of poly A,G(1) and poly U.

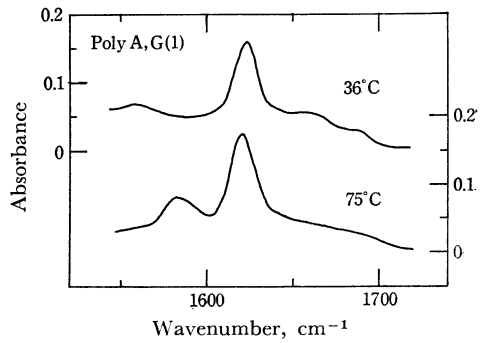

Fig. 12. Infrared absorption spectrum of poly A,G(1) in D_2O + 0.2 M NaCl + 0.01 M Nacacodylate buffer, pD 7.0.
Approximate concentration of poly A,G(1) is 0.02 M.

15) See, for example, M. Tsuboi, Applied Spectroscopy Reviews (Marcel Dekker), Vol. 3, 45—90 (1969).
16) H. T. Miles and J. Frazier, *Biochem. Biophys. Res. Commun.*, **14**, 21 (1964).
17) S. Higuchi, Doctor Thesis, Tokyo University, 1968.

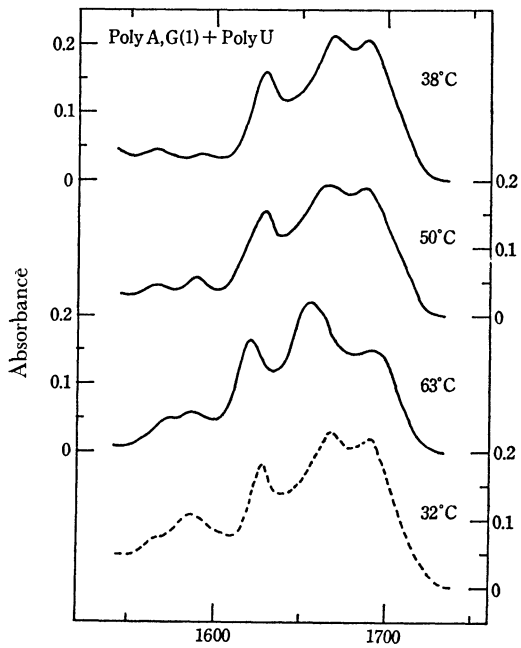

Fig. 13. Infrared absorption spectrum of 1:1 (molar) mixture of poly A,G(1) and poly U.
Solvent: D_2O + 0.2 M NaCl + 0.01 M Na-cacodylate buffer, pD 7.0. Total concentration of nucleotides: about 0.04 M. The lowest curve was obtained when the solution heated up to 63°C was cooled again to 32°C.

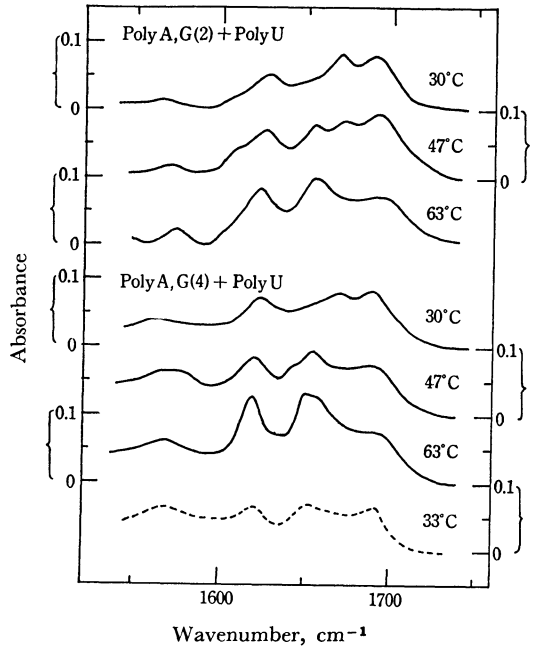

Fig. 14. Infrared absorption spectra of 1:1 (molar) mixtures of poly A,G(2)+poly U and of poly A,G(4)+poly U.
Solvent: D_2O + 0.2 M NaCl + 0.01 M Na-cacodylate buffer, pD 7.0. Total concentration of nucleotide: about 0.02 M. The lowest curve was obtained when the poly A,G(4)+poly U solution heated up to 63°C was cooled again to 33°C.

Therefore, it is evident that 1 : 1 (molar) mixture of poly A,G(1) and poly U forms a double-helical complex, poly A,G(1)–poly U, in 0.2 M Na^+ at 38°C. The ultraviolet (259 mμ) mixing curve shown in Fig. 2 (A) does not show any bend in the vicinity of the mole ratio 1/1, and therefore this might be taken as indicating that a 1 : 1 (molar) mixture of poly A,G(1) and poly U consists of a triple-helical poly A,G(1)–poly U–poly U and single-stranded poly A,G(1) in the solvent with 0.1 M or more Na^+. By means of the infrared absorption measurement, however, this is not found to be actually the case. We consider that here is a double-helical complex, poly A,G(1)–poly U, and that this is not distinguishable from the triple-helix plus single poly A,G(1) by the ultraviolet absorbance at 259 mμ. Similar situation was already found for 1 : 1 (molar) mixture of poly A and poly U in high-salt solution.[12,14,16]

Infrared spectra of 1 : 1 (molar) mixtures of poly A,G(2) & poly U and of poly A,G(4) & poly U are given in Fig. 14. Both of these show the characteristic bands (1690, 1670, and 1630 cm^{-1}) of Watson-Crick type A···U structure in 0.2 M Na^+ and at 30°C.

The melting process of these double-helical complex, poly A,G–poly U, was examined by means of the infrared absorption measurement, and the results are shown also in Figs. 13 and 14. At this salt concentration (0.2 M Na^+), the double-helical structure seems to decompose directly into single-stranded poly A,G and poly U. These give strong bands at 1692 cm^{-1} (C_2=O stretching of uracil), 1657 cm^{-1} (C_4=O stretching of uracil), and 1621 cm^{-1} (double-bonds stretching of adenine). On lowering the temperature of the solution again to 32 or 33°C, the A···U base-pair is reformed for poly A,G(1) + poly U (see the lowest curve in Fig. 13), but not for poly A, G (4) + poly U (lowest curve in Fig. 14).

The guanine residues involved in the copolymer should give absorptions in the 1600—1550 cm^{-1} region. There is certainly an absorption band observed in this spectral region in every spectrum of poly A, G (Fig. 12) and of poly A, G + poly U (Figs. 13 and 14). It appears at 1565 cm^{-1} for lower temperatures, and on elevating the temperature it is shifted to higher frequency (at about 1590 cm^{-1}). On lowering the temperature, however, the band does not come back to 1565 cm^{-1} even when the absorption bands (at 1689, 1671, and 1630 cm^{-1}) due to the A···U base-pair are restored (see the lowest curve in Fig. 13). This fact shows that the environment of the G residues

changes on breaking down the double-helical structure of the complex molecule and that this change is not easily reversed even when the A···U base-pairs are reformed. No interpretation however is yet given of the spectral change (1565→1590 cm^{-1}).

Discussion

Present ultraviolet and infrared spectroscopic data are well explained by postulating the following items:

(1) In the solvent with 0.1 M or more Na^+, both of the double-helical complex, poly A,G–poly U, with the Watson-Crick type A···U base-pair and the triple-helical complex, poly A,G–poly U–poly U, with the A:$\begin{matrix}\cdot\cdot U\\ \cdot\cdot U\end{matrix}$ type inter-base binding are to be formed at 10°C. What are formed depend upon the mol % of poly U in the solution:

0 to 50%	single poly A,G and double helix
50%	double-helix
50 to 66%	double-helix and triple-helix
66%	triple-helix
66 to 100%	triple-helix and single poly U.

(2) On heating such a solution, the triple-helical complex first decomposes into double-helix and single poly U, and then at a higher temperature melts into three single-stranded random coils.

(3) In the solvent with Na^+ concentration as low as 0.01 M, only double-helical complex is formed at 10°C.

(4) A guanine residue in the copolymer poly A,G remains within the helical structure when its neighbors are mostly adenine residues. Otherwise, it rotates out from the helix so that the complementary A···U binding can be formed in the subsequent region of the polynucleotide chains.

What is described in item (4) here may be related with the "wobble hypothesis" of Crick.[18] It has now been established that three base-pairs are formed between the "codon" of a messenger RNA and the "anticodon" of a transfer RNA in a certain step of translation of a genetic code in a biological system. Crick[18] suggested that while the standard base-pairs (A···U and G···C) are used rather strictly in the first two positions of the three codon-anticodon pairings, there may be some wobble in the pairing of the third base. He pointed out several possible base-pairs for the third position, and among them the G···U pair is included. It may also be pointed out here that guanine and uracil residues are often located in a primary structure of a transfer RNA molecule at a position in which we usually postulate a strong A···U or G···C base pair in other transfer RNA molecules.[19] Our present experiment indicated that such a "G···U base-pair" certainly takes place if there are a number of standard base-pairs formed in its neighborhood. Our experiment also indicated, however, that such "G···U base-pairs" are not stable enough to support a helical structure by themselves.

We wish to express our thanks to Professor Makoto Ishimoto and Mr. Kaoru Saigo for their instruction in our preparation of polynucleotides by the use of the enzyme from *Micrococcus lysodeikticus*, to Professor Sanae Mii for her providing wet cells of *Azotobacter vinelandii*, and to Dr. Kimiko Matsuo for her valuable advices. This work was supported by a grant from the Ministry of Education of Japan and by a grant from the United States Public Health Service (GM 10024—06).

18) F. H. C. Crick, *J. Mol. Biol.*, **19**, 548 (1966).
19) See, for example, G. R. Philipps, *Nature*, **223**, 374 (1969).