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DEMONSTRATION OF G • U WOBBLE BASE PAIRS BY RAMAN AND IR SPECTROSCOPY

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When guanine and uracil form hydrogen bonds in the pairing scheme first proposed by Crick one would expect that poly(A,G) will form an unperturbed double helix with poly U at room temperature in a dilute electrolyte solution (0.1 M NaCl). We have demonstrated by Raman- and IR-spectroscopy that the secondary structure of poly(A,G) • poly U is very similar to the structure of poly A • poly U; only the thermal stability of the double helix seems slightly lower than the stability of poly A • poly U, whereas the average helix length is unaffected by the dispersed G • U base pairs. From our input ratio of guanine and adenine we estimate that about every fourth base pair is a wobble pair.

1. Introduction

Recently Kearns and coworkers [2] have examined the behavior of poly d(G-T) by the aid of NMR spectroscopy and ethidium bromide binding since this was the one system where the evidence for formation of wobble pairs was strongest. The results of these NMR studies confirm the base pairing scheme proposed earlier by Lezius and Domin [3]. Nevertheless the occurrence of the G • T wobble base pairs in poly d(G-T) does by no means prove the occurrence of a G • U pair in a Watson Crick type double helix. Therefore we have investigated this matter further with an other model system.

Poly(A,G) is assumed to form a double helix with poly U, where G • U base pairs may be inserted into a poly A • poly U "host helix". This approach has the advantage that the A • U base pairs account for the geometry and stability of the ordered structure, while the G • U base pairs can adjust to this linear lattice. Vibrational spectroscopy is used as the tool to detect the participation of the C=O group of guanine in a hydrogen bonding scheme [4]. UV melting curves are recorded as function of the temperature at different ionic strengths at 257 nm and at 280 nm. The second wave length was chosen to examine the possibility of a triple helix formation at elevated ionic strength [5]. From the slope of the UV melting curve at T_m the apparent enthalpy of transition (ΔH_{vH}) can be calculated. The comparison of the ΔH_{vH} and the true transition enthalpy ΔH for one A • U base pair allows to estimate the helix length which can serve as a measure for the conservation of the helical parameters in the poly(A,G) • poly U double helix [6].

2. Material and methods

Poly U, ADP, GDP and polynucleotide phosphorylase were purchased from Boehringer, Mannheim. Serum albumin was purchased from Serva, Heidelberg. Biogel P-30 was purchased from Bio Rad, München. In a standard polymerization assay 0.2 mmol ADP and 0.043 mmol GDP were dissolved in 4 ml 0.2 M tris buffer, pH 8.7, containing 15 mM $MgCl_2$, 0.25 mM EDTA, 30 units polynucleotide phosphorylase and 1 mg serum albumin. The reaction mixture was allowed to stand for 5 h at 40°C and then cooled. Proteins were extracted five times with $CHCl_3$ and once by isoamyl alcohol [7]. Poly (A,G) was purified by chromatography on a Bio-Gel P-30 column.

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2.1. IR spectroscopy

The infrared measurements were carried out with a Perkin-Elmer 325 spectrometer according to the method used by Schernau and Ackermann [8] in D_2O solution. The spectra were digitized and extinction coefficients were calculated in steps of 2 cm^{-1}. The molar ratio of A and G in poly (A,G) was determined to be 3.8 and the UV extinction coefficient was calculated from the infrared spectrum of the dinucleotide ApA and of the mononucleotide GMP. Poly (A,G) and poly U were mixed in equimolar amounts based on the molar absorbancies of the constituents. The solution contained about 0.03 M base pairs of the polymer, 0.1 M NaCl and 0.05 M Tris-HCl, pH 7.5.

2.2. Raman spectroscopy

The Raman apparatus used throughout these measurements has been described previously [9,10]. The 5145 Å exciting line of a CR-8 argon ion laser yielded about 1 Watt in the sample. The spectra were recorded in the 400–1800 cm^{-1} region at a spectral slit width of 4 cm^{-1}: The stepping motor of the Coderg PHO spectrometer was controlled by a PDP-8/1 computer and the spectral data of each scan were accumulated in the core memory.

In order to improve the signal-to-noise ratio, we used the time-averaging technique with more than 100 runs for each spectrum. Because the carbonyl bands of G and U, which are probably affected by hydrogen bonding, are largely obscured by the water bending mode in H_2O solution, we subtracted the solvent spectrum recorded in the same manner in the 1500–1750 cm^{-1}. To clarify the role of the G within the ordered structure, we also subtracted the spectrum of the poly A • poly U double helix in this frequency region. Details of our subtraction technique will be described elsewhere [11].

The polymer concentrations in the H_2O and the D_2O solutions were the same as those in the IR measurements. All UV measurements were made with the help of a Pye Unicam Sp 1800 spectrometer. Details of this procedure were published earlier [12].

3. Results

3.1. UV spectroscopy

The absorption of the complexed poly (A,G) • poly U was recorded as a function of the temperature at two different wave lengths, namely 257 nm and 280 nm, under various salt conditions to compare the results with the melting of poly A • poly U.

The absorption at 257 nm versus temperature resembles very much the curve for the pure poly A • poly U [13]. The plot at 280 nm recorded with poly (A,G) • poly U in 1 M salt does not show any hypochromic effect at moderate temperatures due to a triple helix formation followed by a hyperchromic effect due to the transition of this helix to a random coil. Only a single hyperchromic effect due to the double helix coil transition is exhibited. For poly (A,G) • poly U the formation of a triple helix can be excluded. Poly (A,G) • poly U undergoes a reversible helix coil transition at a slightly lower transition temperature than poly A • poly U. The ionic strength dependence of the transition temperature corresponds to the poly A • poly U system. In both cases the slope $dT_m/d(-\log C_{Na^+})$ equals –23°C for the linear part of the T_m versus $-\log c_{Na^+}$ plot. In our case we took the total excess electrolyte content for Na^+ added as NaCl to the polynucleotide solution. The slope $dT_m/d(-\log c_{Na^+})$ corresponds to the results of Gruenwedel [14] for double helical calf thymus DNA. The transition interval is slightly increased as compared to poly A • poly U. This can either be due to a smaller degree of polymerization of the poly (A,G) or to the smaller thermal stability of the G • U pairs within the poly A • U “host helix”. The slope of the absorbancy versus temperature curve can be utilized to calculate the apparent transition enthalpy ΔH_{vH} [15]. The values of ΔH_{vH} vary from 96 to 120 kcal per mole as a function of T_m. If we assume that the transition enthalpy ΔH of a G • U base pair is only slightly lower as of an A • U [13] base pair, the average number of base pairs undergoing the helix coil transition at the same time results to 12 at low ionic strength and increases to at least 15 at high ionic strength.

3.2. IR spectroscopy

Fig. 1 shows the infrared spectrum from 1750 cm^{-1}

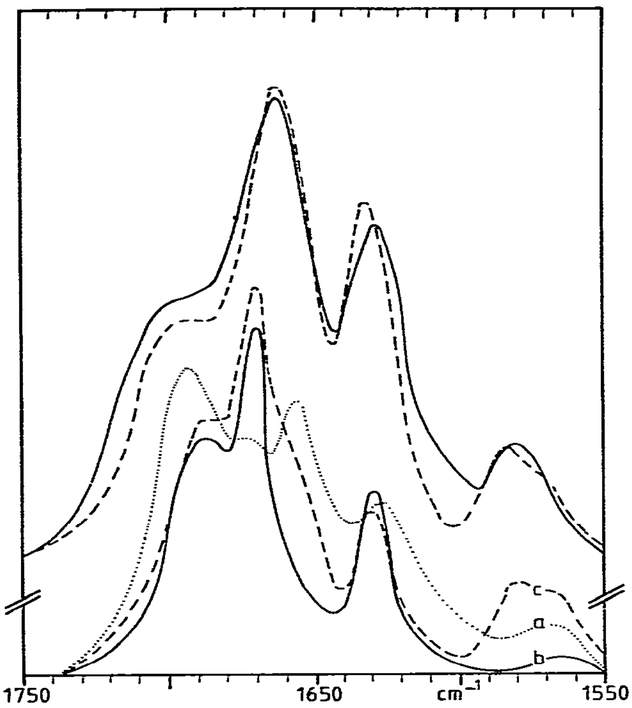

Fig. 1. Lower part: IR spectrum of a) poly (A,G) • poly U (. . .), b) poly A • poly U (——), c) poly A • poly U (0.8) + GMP (0.1) + UpU (0.1) (- - -) at room temperature in the region 1550–1750 cm^{-1}. Upper part: IR spectrum of a) poly (A,G) + poly U (——), b) UpU (0.5) + ApA (0.4) + GMP (0.1) (- - -) at 70°C.

to 1550 cm^{-1} of the poly (A,G) • poly U complex at 15°C (a). This interval was chosen because it covers the strongest bands of nucleotides in D_2O solution. This spectrum differs significantly from the spectrum of poly A • poly U mixed with 10% GMP and 10% UpU (c), which would be expected for a double helix with all adenine residues bound in a Watson Crick type H-bonding scheme and all guanine residues and the remaining uracil residues exposed to the solvent as in a randomly coiled single strand. There are two intense bands with a maximum at 1695 cm^{-1} and at 1656 cm^{-1} in (a) whereas the intense band characteristic for poly A • poly U at 1672 cm^{-1} has almost disappeared and only a weak band at 1674 cm^{-1} is to be seen. The obvious loss of intensity can be explained if we assume that in agreement with poly G • poly C [4] an in-plain-coupling takes place also in the GU pairs which results in a splitting of the band. In poly (A,G) • poly U only one band at 1568 cm^{-1} occurs in the place of the more intense bands of GMP at 1577 cm^{-1} and 1565 cm^{-1}. Upon heating the solution to 70° the ordered secondary structure is broken down and this band at 1577 cm^{-1} increases in intensity and the maximum shifts to 1575 cm^{-1}. This is the same behavior as is shown for guanine residues in Watson Crick type bound G • C base pairs [16].

3.3. *Raman spectroscopy*

Original Raman spectra of poly (A,G) • poly U dissolved in H_2O and D_2O at 5°C are presented in figs. 2 and 3. Observed bands (frequencies and intensities) are given in table 1. A comparison of the poly (A,G) • poly U spectra with those of the poly A • poly U double helical complex, first presented by Small and Peticolas [17], shows that there are striking similarities in the spectra of these two complexes. The band at 814 cm^{-1} in the spectrum of the H_2O solution, and 810 cm^{-1} in the spectrum of the D_2O solution respectively, attributed to the sugar-phosphate-diester stretch of the A form of polynucleotides [18], indicates that poly (A,G) • poly U forms an unperturbed double helical complex. In addition, the positions and relative intensities of almost all the bands in the low temperature spectrum of poly A • poly U are also observed in the poly (A,G) • poly U spectrum.

The most interesting results are expected from those bands related to hydrogen bond formation. The occurrence of G • U base pairs within the poly A • poly U "host helix" can be monitored especially by the C=O stretching vibrations of G and U. In A • U base pairs the C(4)=O of U is engaged in a hydrogen bond to the N(6)H_2 group of adenine, while in G • U pairs the C(2)=O of U is supposed to bind to the N(2)–H_2-group of guanine [19]. From this we expect that part of the signal assigned to the C(4)=O groups of U should be found at the position as in the coiled form of poly U. For this reason, the section of the spectra between 1500 cm^{-1} and 1750 cm^{-1} was recorded again very carefully to give a close view at the bands assigned to the different C=O groups.

Fig. 4 shows the spectra of poly (A,G) • poly U in D_2O at 5°C and 60°C and, in addition, the difference of the spectra of the double helical complexes poly

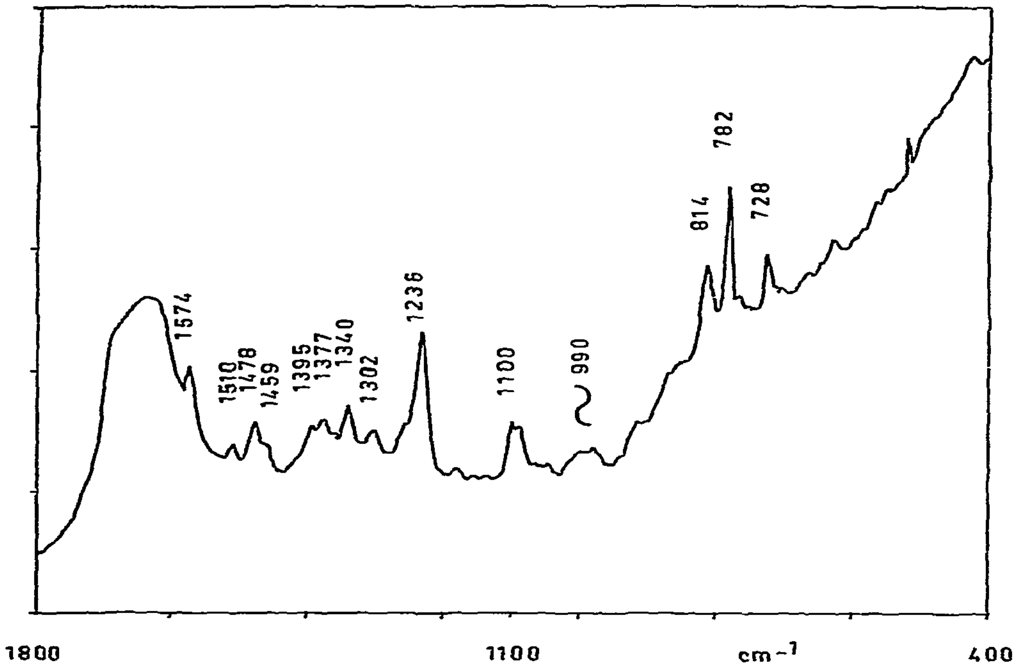

Fig. 2. Raman spectrum of 0.03 M poly (A,G) • poly U in H_2O at 5°C in the region 400–1800 cm^{-1}.

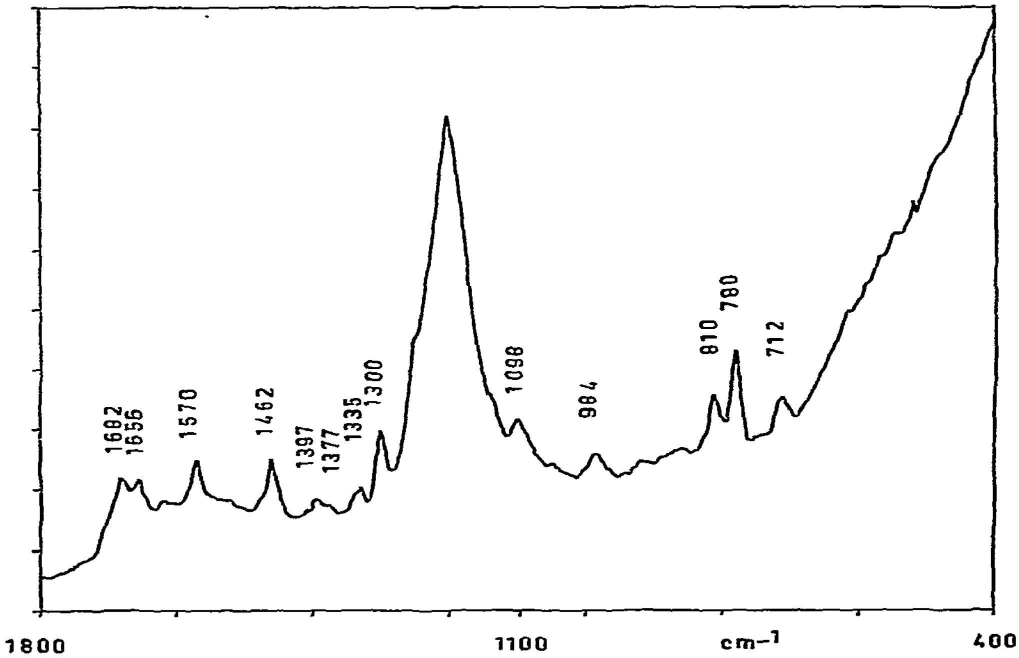

Fig. 3. Raman spectrum of 0.03 M poly (A,G) • poly U in D_2O at 5°C in the region 400–1800 cm^{-1}.

Table 1
Raman frequencies of the poly (A,G) • poly U helix *

Frequencies (cm^{-1})		Assignments
H_2O solution	D_2O solution	
728 m	712 m	A
782 s	780 s	U
814 m	810 m	O–P–O-sym. stretch
990 w	984 w	ribose
1100 m	1098 m	PO_2-sym. stretch
1236 s		U
1302 w	1300 m	A
1340 m	1335 w	A
1377 w	1377 w	A
1395 w	1397 w	U
1459 w(s)	1462 m	ribose
1478 m		A,G
1510 w		A
1574 m	1570 m	A,G
1617 w		A,G,U C=C stretch
	1656 m	mainly U C=O stretch
	1682 m	
1690 s		
	1705 w(s)	G C=O stretch
1725 w		G C=O stretch

* A, G and U indicate vibrations characteristic of the adenine, guanine or uracil ring respectively; w, m and s indicate weak, medium and strong respectively, S denotes shoulder.

(A,G) • poly U and poly A • poly U. One can see that the 60°C spectrum displays the usual C=O bands of uracil at 1758 cm^{-1} and about 1692 cm^{-1} in D_2O solution in the absence of any ordered structure. The guanine C=O stretching vibration at about 1670 cm^{-1} [20] gives only a very small contribution to the spectrum in this region, as the uracil C=O bands are usually much more intense than that of guanine and as there is a four fold molar excess of uracil over guanine. In the low temperature spectrum there are bands at 1656 cm^{-1} and 1682 cm^{-1} and in addition a shoulder at about 1705 cm^{-1}. In the case of the poly A • poly U double helix there is only one band at 1681 cm^{-1} [21]. We assume that this band is due to the stretching vibration of the C(4)=O group of uracil involved in a H-bond to the N(6)-H_2 group of adenine. The finding that there is still a band at 1656 cm^{-1} in the low temperature spectrum of poly (A,G) • poly U therefore indicates that there are contributions of solvated C(4)=O groups of uracil in this complex. This band appears even more pronounced after subtraction of the poly A • poly U spectrum.

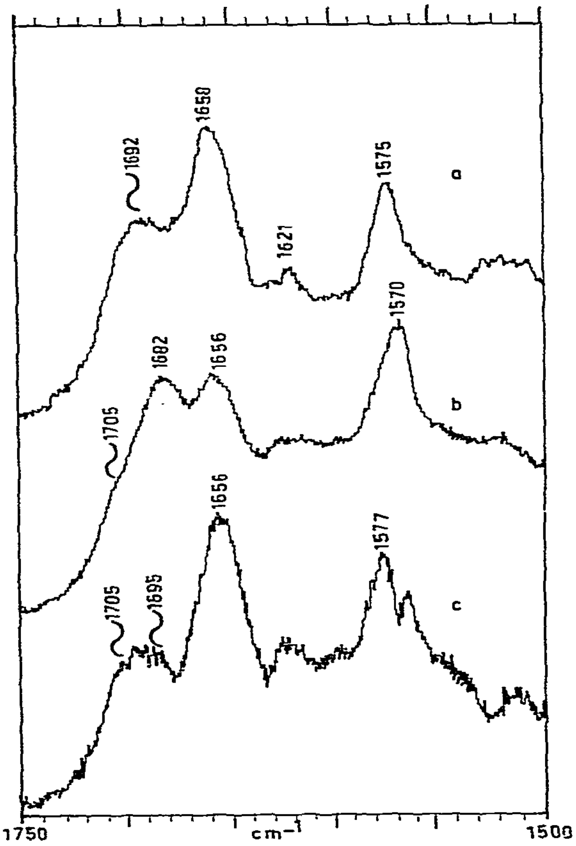

Fig. 4. Raman spectra of poly (A,G) • poly U in D_2O at 60°C (a) and 5°C (b) in the region 1500–1750 cm^{-1}. Difference of the spectra of the double helical complexes poly (A,G) • poly U and poly A • poly U (c) at 5°C.

In H_2O solution the carbonyl bands cannot be observed unless one subtracts the Raman scattering of the solvent water. In fig. 5 "waterfree" spectra of poly (A,G) • poly U at 5°C and 60°C in the region 1500–1750 cm^{-1} are shown. As in the case of the D_2O solution, we computed the difference spectrum of the double helical complexes. It may readily be seen that there is a big band at 1687 cm^{-1} in the 60°C spectrum which is a superposition of the two C=O stretching modes of uracil and of the C=O stretching mode of guanine. It was a surprise to us that

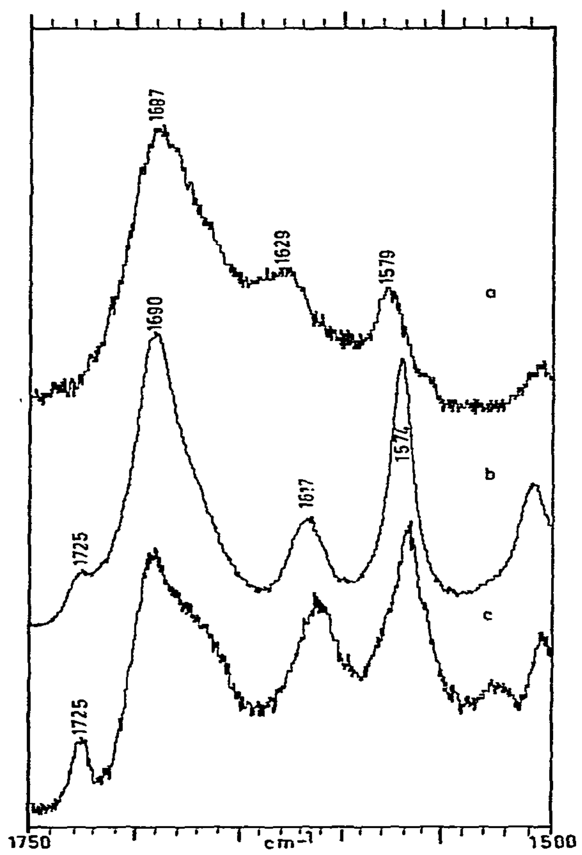

Fig. 5. Raman spectra of poly (A,G) • poly U in H_2O with the solvent subtracted out at 60°C (a) and 5°C (b) in the region 1500–1750 cm^{-1}. Difference of the spectra of the double helical complexes poly (A,G) • poly U and poly A • poly U (c) at 5°C.

also at room temperature all C=O stretching modes fall into the same frequency region and overlap almost completely in H_2O solution. Little can be said about the fraction of hydrogen bonding to the different C=O groups from these spectra. A explanation of the different shifts and couplings in plain and along the helix axis is beyond the scope of this experimental work. Yet, one can see that there appears a new band at 1725 cm^{-1} in the 5°C spectrum. The appearance of this new band is even more pronounced in the difference spectrum of the two double helical complexes. From our observation that in the spectra of various model compounds containing guanine in an ordered structure [22] a band at about 1725 cm^{-1} is present, we conclude that this band indicates the involvement of the C=O group of guanine in the H-bonds of the poly (A,G) • poly U double helix. In D_2O solution, there often appears a band around 1700 cm^{-1}, if guanine is engaged in base pairing [23]. The shoulder at 1705 cm^{-1} in the D_2O spectrum can be taken as a hint for guanine base pairing in poly (A,G) • poly U.

4. Discussion

To obtain information regarding the base pairing scheme in the different parts of the tRNA cloverleaf structure the NMR spectra of various tRNAs were examined. If the ring nitrogen protons from both G and U are involved in NH ... O H-bonds in the NMR spectrum two resonances are expected somewhere between 10 and 12 ppm due to the formation of a wobble base pair. These resonances are found e.g. in the case of $tRNA^{Phe}$ [24]. Recently Kearns et al. have shown that in double stranded poly d(G-T) the same chemical shifts occur in the case of a G • T wobble pair.
To reflect the situation of a GU base pair in the middle of a double helix of regular Watson Crick base pairs we investigated the interaction of poly (A,G) with poly U at pH 7.0 and 0.1 M Na^+. The input ratio of A to G was 4 to ensure on one hand that we have sufficient adenine residues in a stack to serve as a nucleation center for the beginning of a short poly A • poly U double helix even if G does not interact with the opposite U. In this case we would expect a defective helix with small stretches of A • U base pairs interrupted by small loops. The average helix length would be 4 base pairs. The transition temperature T_m would be below room temperature and the cooperativity would be very low, i.e. the transition interval would be extremely broad. The experimental results of the melting curves are contradicting this assumption. The transition temperature is only slightly lowered as compared to the "pure" poly A • poly U system and the average helix length is about 15 bp. These results are in accordance with the assumption of GU wobble base pairs inserted into a poly A • poly U "host helix". The thermal stability and the geometry of this helix seems to be not much affected by the "guest base pairs", leading to the conclusion that they occupy a

very similar geometry. This possibility was discussed earlier by Arnott [25]. On the other hand the fraction of the guanine residues in the poly A strand is high enough to account for about 20 percent GU base pairs. If the scheme of H-bonds between G and U differs from the scheme between A and U there should be sufficient spectroscopic evidence to show the presence of these H-bonds besides the H-bonds of the AU base pairs by means of Raman and IR spectroscopy. The IR spectrum of poly (A,G) • poly U at 70°C (cf. fig. 1) in the range 1550–1750 cm^{-1} reflects the situation of a completely disordered conformation of the polymer chains. This spectrum can be calculated by adding up the known spectra of UpU, ApA and GMP in the same range. The agreement of the two spectra is good enough to allow the conclusion that there are no interchain or intrachain interactions in the polymer strands, for the positions of the bands assigned to the different C=O groups are identical with those of solvated C=O groups of the monomers. The comparison of the poly (A,G) • poly U spectrum at room temperature with the spectrum of poly A • poly U and free GMP and UpU respectively shows that the experimental spectrum differs drastically from the model spectra. The calculated spectrum was introduced to illustrate how the spectrum of a defective helix with the G and the opposite U looped out would look like. This situation is by no means supported by the experimental results. The strong band at 1695 cm^{-1} is shown when guanine is hydrogen bonded via Watson Crick H-bonds to another nucleotide as in poly G • poly C. Unfortunately it is impossible to assigne the band to an individual C=O group of guanine or uracil, because the coupling of these vibrations in the plane of a base pair and in the direction of the helix axis is so pronounced that all the C=O bands are superimposed. From the infrared spectrum we conclude that guanine participates in a base pairing via H-bonds.

This conclusion is also supported by the results of the Raman spectra in D_2O and in H_2O. The band at 814 cm^{-1} is assigned to the presence of the A form of a polynucleotide double helix as in ordinary poly A • poly U. Viewing the spectral range about the C=O bands (1500 cm^{-1} to 1750 cm^{-1}) it is obvious that in the poly (A,G) • poly U spectrum there are fundamental differences as compared to the spectrum of poly A • poly U. In the latter case there is only one strong band at 1681 cm^{-1} in the D_2O spectrum at room temperature. This band contains the contributions from the free C(2)=O band of uracil and from the H-bonded C(4)=O band of uracil. Tsuboi [26] has proposed that there is also some contribution from coupling between the different C=O groups. In the spectrum recorded above the transition temperature of the double helix this single band is replaced by two bands at 1698 cm^{-1} and 1660 cm^{-1}. The first band is assigned to the C(2)=O and the second band is assigned to the C(4)=O of uracil [26]. The Raman spectrum of poly (A,G) • poly U at room temperature exhibits two bands. The band at 1682 cm^{-1} agrees with the band in the spectrum of poly A • poly U. The second band at 1656 cm^{-1} indicates the presence of a fraction of free C(4)=O of uracil due to U residues which form wobble pairs with guanine. In the pairing scheme of the wobble pairs (cf. fig. 6) the C(2)=O group is hydrogen bonded and the C(4)=O group is free. There is also a small shoulder at 1705 cm^{-1} which corresponds to the small peak at 1724 cm^{-1} in the H_2O spectrum. This peak shows always up in the spectra of model compounds which contain guanine residues in a base pairing scheme. Upon heating beyond the transition temperature this small peak disappears. The band at 1682 cm^{-1} is shifted to 1692 cm^{-1} without changing the intensity very much, and the band at 1656 cm^{-1} is strongly enhanced without changing the position. The spectrum of the disordered structure of poly (A,G) • poly U approaches the spectrum of disordered poly A and poly U. Conclusion: The Raman spectra at low temperature can be interpreted if we assume that in the A • U pairs the C(4)=O group is bonded and the C(2)=O group is

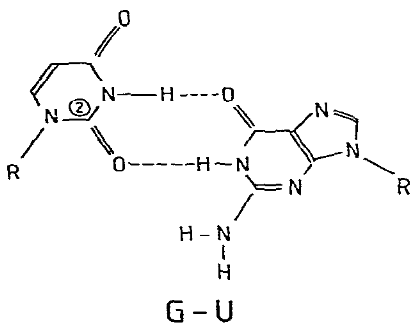

Fig. 6. Pairing scheme for G and U as proposed by F. Crick [1].

free while in the G · U pairs the C(2)=O of uracil and the C(4)=O of guanine participate in a N–H...O H-bond while the C(4)=O group of uracil remains unbonded. The presence of the line at 1725 cm^{-1} shows that guanine is hydrogen bonded but we have no way to assigne the band to a certain C=O group. The line at 814 cm^{-1} confirms the existence of an A form double helix. All arguments presented here allow the conclusion that G · U base pairs are formed and that these base pairs fit into a double helix of poly A · poly U.

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