Raman Spectrum of a Transfer RNA

Abstract. *Raman spectrum of purified formylmethionine transfer RNA from* Escherichia coli *has been observed in its aqueous solution. In the 1800 to 200 cm^{-1} range, about 30 Raman lines are found, each of which can be assigned to one of the constituent nucleotide residues. From the positions and intensities of the lines, information on the intramolecular environments of these base residues can be obtained.*

The purpose of this report is to present a Raman spectrum of a purified transfer ribonucleic acid (tRNA), and then to demonstrate the extent to which the vibrational states of each base residue are perturbed in the secondary structure of the tRNA molecule.

Formylmethionine tRNA from *Escherichia coli* was purified as described (*1*). The tRNA was precipitated by ethanol and dried, and about 10 mg was used to prepare a 0.2-ml aqueous solution, 5 percent in tRNA. The solvent was 0.1*M* NaCl, 0.01*M* tris [tris-(hydroxymethyl)aminomethane] buffer, *p*H 7.5. This solution was placed in a cylindrical glass vessel (2 mm inside diameter by 15 mm length). A JRS-U1 Raman spectrophotometer (Japan Electron Optics) was used. For excitation, we used the 5145-Å line of an argon ion laser (about 240 mw, measured at the sample) instead of the 4880-Å line, because the latter caused a much greater background emission. The spectrum at the top of Fig. 1 was obtained with a 10-cm^{-1} slit width. About 30 Raman lines are observed in the 1800 to 200 cm^{-1} range, and their frequencies are given in Fig. 2.

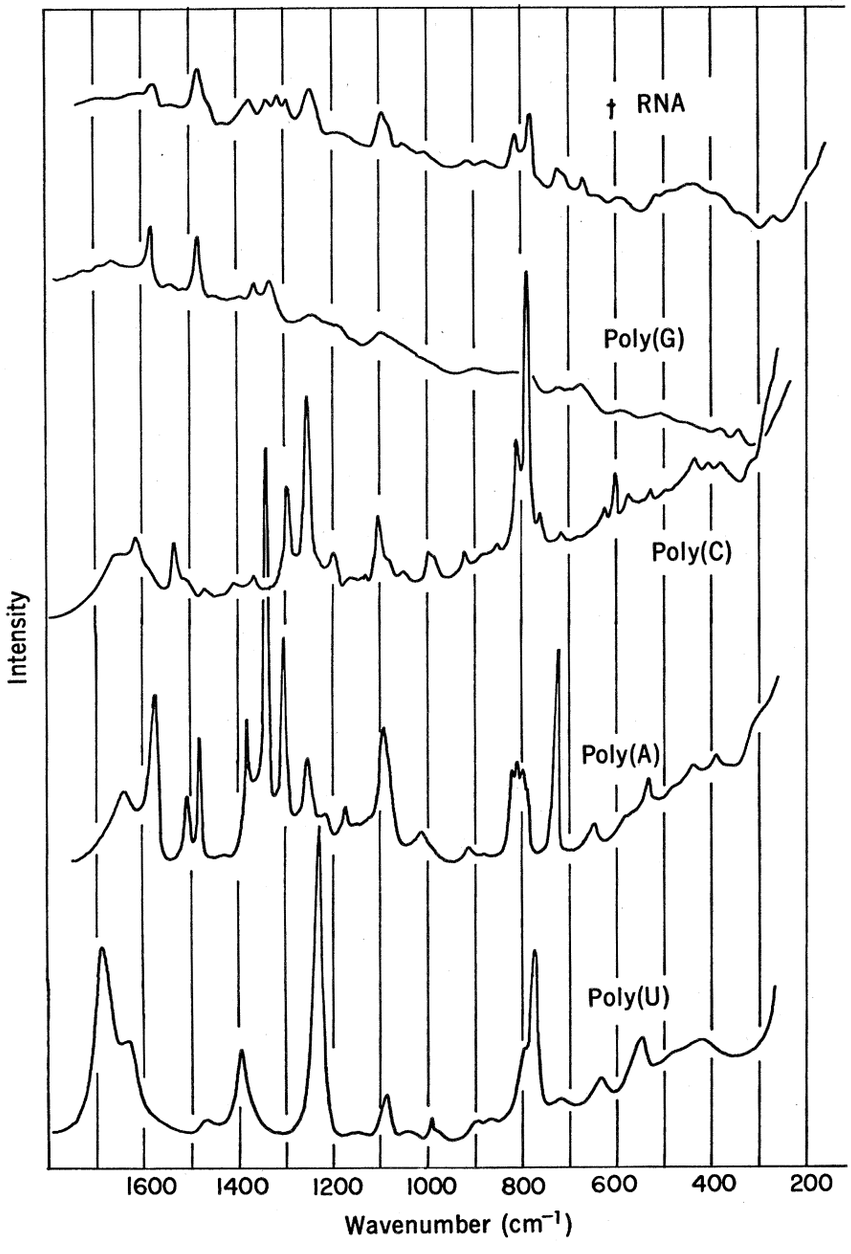

Fig. 1. Raman spectra of formylmethionine tRNA of *Escherichia coli*, poly(G), poly(C), poly(A), and poly(U) in aqueous solutions at *p*H 7.5 at room temperature.

The chemical structure of formylmethionine tRNA from *E. coli* is now well established (*2*). It is a single polyribonucleotide chain with 77 nucleotide residues, of which 24 are those of guanylic acid, 25 cytidylic acid (including one 2′-*O*-methyl derivative), 15 adenylic acid, and 8 uridylic acid; the remaining 5 are residues of odd nucleotides all different from one another. In interpreting the Raman spectrum of such a nucleic acid, it is appropriate to compare it with the spectra of the homopolymers of the four regular ribonucleotides: polyriboguanylic acid, poly(G); polyribocytidylic acid, poly(C); polyriboadenylic acid, poly(A); and polyribouridylic acid, poly(U). The chemical structure of each nucleotide residue is exactly the same in the homopolymer as in tRNA. Differences in the Raman spectra of a nucleotide residue in the homopolymer and in tRNA are attributable to differences in their secondary structures. (Differences caused by vibrational coupling between the adjacent nucleotide residues would be negligibly small.) The Raman spectra we observed for the four homopolymers at *p*H 7.5 are given in Fig. 1. In general they are in good agreement with those observed by others (*3*), but in some ways our spectra show higher resolution. For our present purpose, we constructed synthetic spectrum from the homopolymer spectra in the following way.

Every polyribonucleotide has a Raman line of medium intensity at 1100 cm^{-1}. It has been established that this Raman line is caused by the symmetric stretching vibration of the PO_2^- group (*4, 5*). The absolute intensity of such a Raman line probably remains constant for any nucleotide residue; therefore we first normalized the intensities of the homopolymer Raman lines so that the intensity of the 1100 cm^{-1} line is 1.0_0. The observed frequencies and normalized intensities of the Raman lines are given in Table 1. Next, we multi-

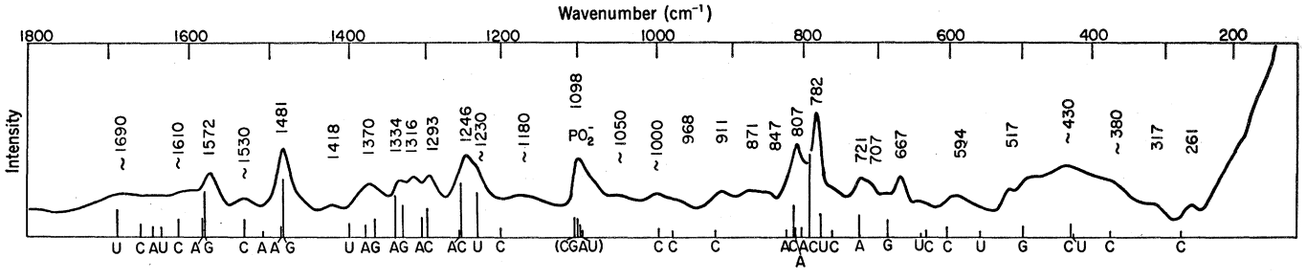

Fig. 2. The continuous curve represents the observed Raman spectrum of formylmethionine tRNA (spectrum at top of Fig. 1, but with background emission subtracted). The vertical lines are the positions and intensities of the Raman lines in a synthetic spectrum, which is a linear combination of the "normalized" Raman spectra (given in Table 1) of the four homopolymers; coefficients are proportional to the base contents in tRNA.

plied these intensities by a factor corresponding to the tRNA composition; the factor is 24 for poly(G), 25 for poly(C), 15 for poly(A), and 8 for poly(U). The contributions from the odd nucleotides are sufficiently small and have been neglected. The positions and intensities of the Raman lines in the synthetic spectrum are shown in Fig. 2, with their origins [guanine (G), cytosine (C), adenine (A), and uracil (U)]. Most of these Raman lines are ascribed to vibrations localized in the base residues of the nucleotides; the Raman lines due to the vibrations of the ribose part would be much weaker (*6*), and would not have such a variety of positions and intensities.

The agreement of this synthetic spectrum with the observed tRNA spectrum (Fig. 2) is in general so good that assignment of many of the Raman lines of tRNA can be made by comparing the spectra. The Raman lines at 1572 and 1481 cm^{-1} are assigned to the G residue; those at 1530, 1000, 968, 911, 807, and 782 cm^{-1} to the C residue; that at 721 cm^{-1} to the A residue; and a broad peak at about 1690 cm^{-1} and a shoulder at about 1230 cm^{-1} to the U residue. The peak at 1370 cm^{-1} would consist of three unresolved lines due to the U, A, and G residues. Three peaks, at 1334, 1316, and 1293 cm^{-1}, are to be allotted in some way to two A, one G, and one C lines. It appears necessary to assign the peaks at 667, 594, and 707 cm^{-1} to the G, C, and A residues, respectively, although their positions are rather greatly different in the homopolymer and tRNA spectra.

Other differences are found between the synthetic spectrum of the homopolymers and the observed spectrum of tRNA. While the position and intensity of the 1481 cm^{-1} G line of tRNA agree with those of poly(G), those of the 1572 cm^{-1} line do not; the latter is situated at a lower (about 8 cm^{-1}) frequency and is lower in intensity than the corresponding G line in poly(G). Likewise, the G line at 667 cm^{-1}; C lines at 1246, 782, and 594 cm^{-1}; and A lines at 721 and 707 cm^{-1} are all at lower frequencies than the corresponding lines in the homopolymers. The frequencies of stronger Raman lines of G, C, and A residues in the 1350 to 1290 cm^{-1} region are also apparently different in tRNA from those in the homopolymers. In addition, the relative intensities of the 1334, 1316, and 1230 cm^{-1} lines are considerably lower than one would expect from those observed in the homopolymers. Finally, a broad emission in the 500 to 350 cm^{-1} region of the tRNA spectrum does not appear in any of the homopolymers. (It is known that glass gives a broad Raman band in this region. It is not evident, however, that the emission we observed is attributable to the glass cell or to some other glass optical component.)

Each of these spectral differences is interpreted as reflecting a difference in the average environment of the base residue in the tRNA molecule from that in the homopolymer. First, the lowering of a Raman line frequency indicates the lowering of some bond-stretching force constants in the base residue. It is probable that hydrogen bonding between bases (G · C and A · U pairing) (*7*) in tRNA is responsible for such a lowering of force constants. On the other hand, the lowering of the intensity is attributable mostly to the stacking between bases; it is known that the intensities of some Raman lines of the base residues are closely related to their ultraviolet absorption intensities at 2600 Å (*5*, *8*).

To obtain a more detailed correlation of the spectral differences with the secondary structure of a nucleic acid, we are following two additional lines of approach. On the one hand, a normal coordinate treatment of the four base residues has been carried out, and a

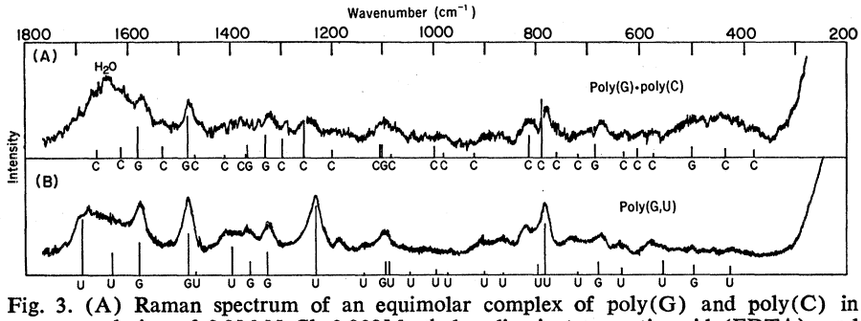

Fig. 3. (A) Raman spectrum of an equimolar complex of poly(G) and poly(C) in aqueous solution of 0.2*M* NaCl, 0.002*M* ethylenediaminetetraacetic acid (EDTA), and 0.01*M* tris buffer, *p*H 7.6. The complex was formed (method given by Dr. G. J. Thomas, Jr., private communication) by dialysis of an equimolar solution for 48 hours at 4°C against 0.01*M* tris buffer, *p*H 8.7, followed by dialysis for 24 hours at 4°C against 0.03*M* NaCl, 0.001*M* EDTA, *p*H 7.3, and precipitation, washing, and drying. The concentration of complex in the sample solution was about 1 percent. This was found to be much more viscous than each of the 5 percent solutions of the component homopolymers, and the G and C residues in the solution are considered to be mostly in the G · C base pairs in a double-helical structure poly(G) · poly(C). (B) Raman spectrum of a copolymer of G and U (1 mole : 1 mole) with a random sequence, in neutral aqueous solution. Verticle lines in A and B give positions and intensities of the Raman lines in the synthetic spectra, which are linear combinations of the "normalized" Raman spectra (given in Table 1) of the homopolymers with coefficients proportional to the base contents in the polynucleotides.

probable normal mode of vibration has been assigned to each strong Raman line. This provides a good means for interpreting the spectral differences. On the other hand, Raman data are being accumulated for a number of oligo- and polynucleotides with known conformations. An examination along this line has suggested, however, that the correlation is not simple. As may be seen in Fig. 3, for example, the frequencies of the G lines at 1580, 1330, and 685 cm^{-1} become lower on going from poly(G) to poly(G) · poly(C), but not as much lower as from poly(G) to the formylmethionine tRNA. In addition, the frequency lowerings of the G lines take place (although only slightly) even on going from poly(G) to poly-(G,U), a random copolymer, which probably has a random coil conformation.

Table 1. Frequencies and normalized intensities of the Raman lines of polyribonucleotides in their H_2O solution. No corrections have been made for the efficiencies of the diffraction gratings and the spectral response of the photomultiplier. Intensities should be multiplied by the correction factors: 0.93 at 500 cm^{-1}, 1.00 at 1100 cm^{-1}, and 1.08 at 1600 cm^{-1}.

Frequency (cm^{-1})	Intensity	Frequency (cm^{-1})	Intensity
Poly (G)		Poly(A)	
500	0.7_5	308	0.1_8
685	1.0_0	391	0.1_8
1100	(1.0_0)	439	0.0_9
1330	1.7_5	537	0.1_8
1365	1.0_0	647	0.1_8
1483	3.2_5	728	2.0_0
1580	2.5_0	804	0.7_3
Poly (C)		814	0.8_2
279	0.3_9	823	0.6_4
318	0.2_3	916	0.1_8
380	0.4_6	1017	0.0_9
436	0.7_7	1098	(1.0_0)
498	0.0_8	1176	0.1_8
525	0.0_8	1216	0.0_9
574	0.2_3	1254	0.5_5
606	0.6_2	1305	1.6_4
632	0.3_9	1340	3.6_4
718	0.2_3	1379	1.0_9
760	0.3_9	1426	0.0_9
791	4.5_4	1484	1.0_9
813	1.7_7	1509	0.4_5
853	0.1_5	1580	1.5_4
921	0.3_1	1644	0.7_3
981	0.3_1	Poly(U)	
999	0.4_6	430	0.5_6
1050	0.0_8	559	1.0_0
1084	0.2_3	640	0.5_6
1104	(1.0_0)	724	0.2_2
1133	0.0_8	786	4.0_0
1160	0.0_8	802	0.7_8
1200	0.4_6	868	0.1_1
1254	2.9_2	906	0.2_2
1297	1.5_4	979	0.1_1
1367	0.2_3	999	0.4_4
1410	0.1_5	1053	0.1_1
1468	0.1_5	1093	(1.0_0)
1532	0.8_5	1142	0.1_1
1613	0.8_5	1234	7.4_5
1660	0.6_2	1400	2.2_2
		1472	0.3_3
		1631	1.6_7
		1689	4.4_4

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Leech-Repellent Property of Eastern Red-Spotted Newts, Notophthalmus viridescens

Abstract. *Eastern red-spotted newts* (Notophthalmus viridescens, *Salamandridae) are rarely attacked by leeches. This protection is not shared by related salamandrids or by* Ambystoma *(Ambystomatidae). Tetrodotoxin is not the repellent. The immunity of* Notophthalmus *to leech parasitism is probably most significant in its aquatic stages, although the terrestrial efts are also protected.*

The toxic properties of skin secretions and eggs of some salamanders have long been known. Most research has been devoted to the biochemistry and pharmacology of the substances involved (*1*), although a few investigators have tried to determine the effects of the toxins on salamanders' natural vertebrate predators (*2–4*). Many members of the family Salamandridae contain tetrodotoxin (tarichatoxin), which is among the most toxic nonprotein substances known (*5*, *6*). Ambystomatid salamanders produce an as-yet-unidentified substance which repels vertebrates (*7*). Although some consideration has been given to the role of these toxins in protecting salamanders from vertebrate predators, their role in discouraging attack by invertebrate predators such as leeches has not been considered.

Eastern red-spotted newts (*Notophthalmus viridescens*), spotted salamanders (*Ambystoma maculatum*), Jefferson's salamanders (*Ambystoma jeffersonianum*), rough-skinned newts (*Taricha granulosa*), California newts (*Taricha torosa*), and crested newts (*Triturus cristatus*) were exposed to attack by leeches in aquariums containing 10 cm of pond water each. Four species of leeches were used. *Batracobdella phalera, Mooreobdella fervida, Haemopis marmorata, and Helobdella stagnalis.* The leeches were collected locally from a pond which is a breeding site for *Notophthalmus* and both species of *Ambystoma*. Each trial included four to six individuals of each salamander species. When leeches began to draw blood from a salamander, they remained attached to the animal for 3 hours or more if they were not disturbed. In each trial the salamanders were examined at intervals of from 1 to 3 hours, the species of leech and its location on the salamanders noted, and the leeches removed.

With a single exception, only *Batracobdella* attacked live salamanders; one *Helobdella* was observed on an *Ambystoma jeffersonianum*. The data from all trials have been combined in Table 1. The surface area for each salamander was calculated from its weight (*8*). The exposure is equal to the total surface area for each species in each trial multiplied by the number of trials. The data were tested by χ^2; the null hypothesis was that attack by leeches would be proportional to exposure for each species. Attacks by leeches on both species of *Ambystoma* and on *Taricha granulosa* were proportional to exposure (P for the null hypothesis $> .05$). *Taricha*