THE DEPENDENCE OF RAMAN SCATTERING ON THE CONFORMATION OF RIBOSOMAL RNA*

G. J. Thomas, Jr.#, G. C. Medeiros#, and K. A. Hartman+

Received June 4, 1971

SUMMARY

The intensities of Raman scattering from certain vibrations of nucleotide residues in 16S and 23S ribosomal RNA are sensitive to changes of RNA conformation with temperature. The intensity effects differ from those exhibited by polynucleotides and may be due to a specific RNA tertiary structure or to a sequence dependence of the Raman hypochromic effect.

Raman spectra, which arise from the change in polarizability which accompanies the vibrations of molecular subgroups, have provided information on the conformation of RNA (1, 2). We report here the Raman spectra of purified 16S RNA and of purified 23S RNA at several temperatures between 17 and 85°C.

The 16S and 23S RNAs were extracted (SDS-phenol method) from 30S and 50S ribosomes of E. coli D_{10}, using standard procedures (3). To obtain Raman spectra, dried RNA was dissolved to 35 mg/ml in D_2O (99.8%, Diaprep, Inc.) containing 0.1% sodium dodecyl sulfate (SDS). Spectra were excited with the 4880A line of an argon ion laser (Carson Laboratories) and recorded at constant temperature on a Spex Model 1401 spectrometer (2, 5).

---

* Supported by grant GB 8150 of the National Science Foundation, by Southeastern Massachusetts University and by the University of Rhode Island.

Department of Chemistry, Southeastern Massachusetts University, North Dartmouth, Massachusetts 02747

+ Department of Bacteriology and Biophysics, University of Rhode Island, Kingston, R. I. 02881.

The Raman spectra of 16S and 23S rRNA show three intensity maxima above 1600 cm^{-1} (1688, 1660, and 1622 cm^{-1}) which arise from the in-plane stretching vibrations of the bases (Fig. 1). As the temperature of the RNA solutions is increased, the scattering envelope of these lines changes, due primarily to the thermal rupture of hydrogen bonds between base-paired double stranded regions of the RNA. A similar change has been observed in the infrared spectrum and provides a basis for the quantitative determination of base pairing in RNA (4).

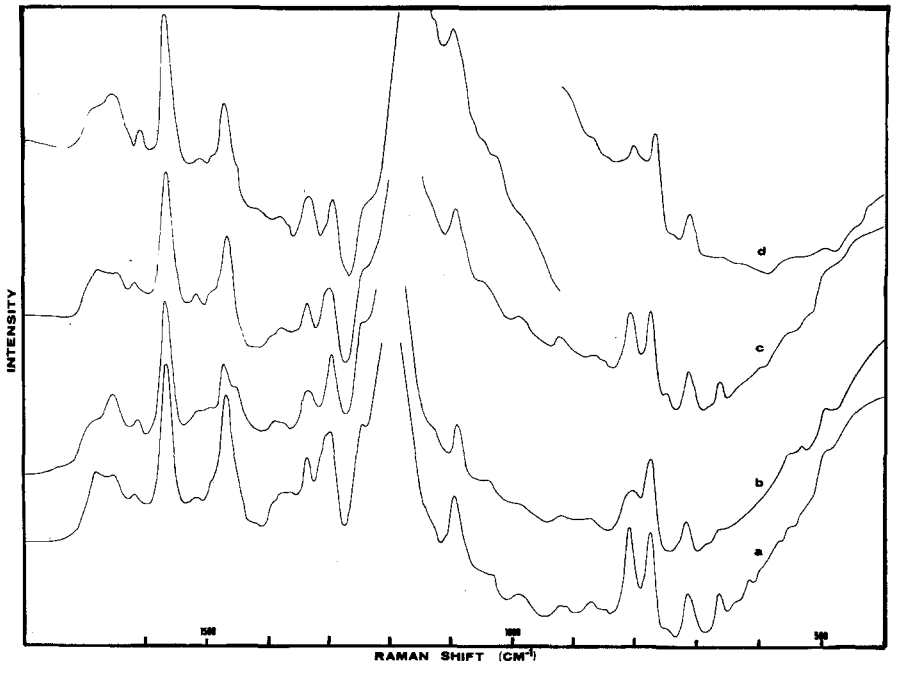

Figure 1: Raman spectra of 16S RNA at (a) 30° and (b) 85°C, and 23S RNA at 30° (c) and 75°C. (d). Scattered intensity (arbitrary units) is recorded vs. Raman shift (cm^{-1}) from Ar 4880 line. Sample concentration is 35 mg/ml D_2O, volume 10 ul, slit width 10 cm^{-1}, scan speed 25 cm^{-1}/min and period 10 sec in each case. The broad intense scattering near 1210 cm^{-1} extending off scale in each spectrum is due to solvent. The additional break in spectrum (d) near 900 cm^{-1} represents a change of baseline. High temperature spectra are uncorrected for solution expansion (approximately 3%).

The scattering line at 815 cm^{-1} in the Raman spectra of 16S and 23S RNA has been assigned to a vibration of the ribose-phosphate linkages (2). As temperature is increased (Fig. 2), the intensity of this line decreases which demonstrates that the orientation of some fraction of the ribose-phosphate linkages is changing as the RNA base pairs are dissociated.

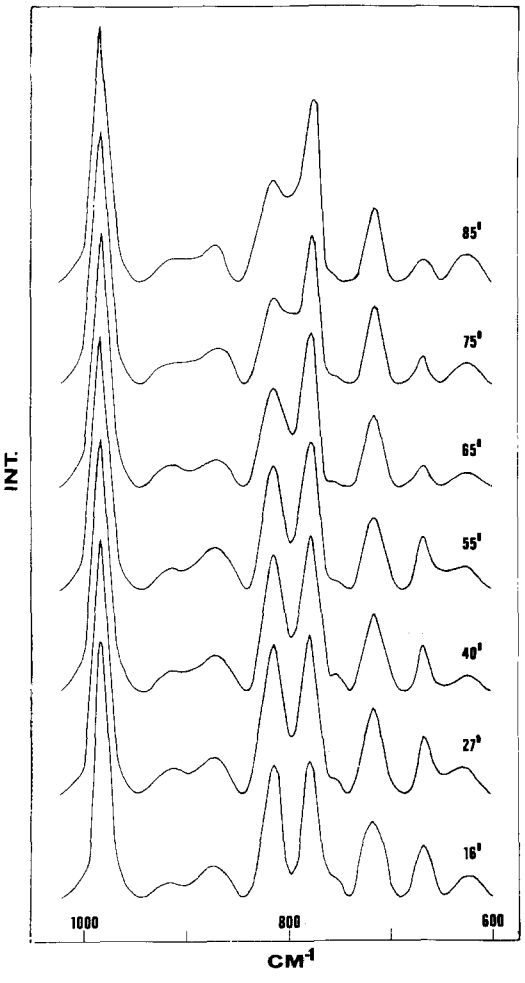

Figure 2: Raman spectra of 16S RNA in the region 1000 - 600 cm^{-1} at several temperatures. Each spectrum is corrected for solvent expansion and normalized to the intensity of the 980 cm^{-1} line of SO_4^{-2} (0.01M Na_2SO_4) added as an internal standard. Other conditions are as in Fig. 1. We have not yet been able to record comparable spectra of 23S RNA because of difficulty in establishing a flat baseline. See, for example, Fig. 1 (d).

The remaining lines in the Raman spectrum are due to planar vibrations of the atoms in the base residues and several lines can be assigned to individual bases. Guanine (G) gives rise to lines at 1480, 1370, and 670 cm^{-1} (2). As the fraction of bases paired and stacked changes with increasing temperature, the intensity of these lines decreases. Adenine (A) residues give rise to a line at 720 cm^{-1} which shows no change in intensity versus temperature. The line observed at 780 cm^{-1}, which increases in intensity with increasing temperature, is assigned to cytosine (C) and uracil (U) residues (Fig. 2, ref. 2).

The interpretation of the intensity data is based on results obtained with homopolynucleotides. Studies on the melting of poly A, poly U and poly C (which have single-stranded stacked configurations) have demonstrated that certain Raman lines (poly A, 725 cm^{-1}*, poly U and poly C, 780 cm^{-1}) show an increase in intensity as the bases unstack with increasing temperature (6). Therefore these Raman lines show a hypochromic effect similar to that observed at 2600A in ultraviolet absorption spectra. Although the effect of melting out stacked regions of poly G has not been observed by Raman spectroscopy, theory predicts that the intensity of the Raman lines from poly G as well as from poly A, poly U and poly C should increase as stacking diminishes (6).

We assume that the hypochromic effect observed and predicted for the homopolynucleotides also obtains for the sequences of the different bases

---

* The ring modes discussed here are largely unaffected by deuteration (2). The Raman lines at 720 cm^{-1} in D_2O and 725 cm^{-1} in H_2O are due to essentially the same ring mode of A. The line at 1345 cm^{-1} (Fig. 1) is also due to A, although erroneously reported as due to U (2). This line for rRNA is unaffected by temperature (Fig. 1) but is hypochromic in single stranded poly A (6).

which are stacked (either single-stranded or double-stranded stacks) in rRNA so that we may predict the amount of stacking of a given base residue from the relative intensity of the appropriate Raman line. Then our intensity data show that as temperature increases, the amount of base pairing diminishes (conventional melting), the fraction of cytosine and uracil residues in stacked configurations (base-paired stacks plus single-stranded stacks) decreases, the amount of A residues in stacked configurations remains constant, and the amount of G residues in stacked configurations increases. At 85°C when virtually all base pairs are broken (conventional melting is complete) there are fewer U and C residues in stacked regions, the same number of A residues in stacked regions, and a greater number of G residues in stacked regions than are found at 30°C, where 60% of the bases participate in base pairing (4).

This result suggests the following as possible structural features of 16S RNA. At 30°C the RNA molecules have many base-paired helical regions which may interact with each other and with single-stranded regions in such a way as to produce a number of single-stranded regions with a constrained configuration in which G and A residues are prevented from assuming the single-stranded stacked configuration which would be energetically favorable in the absence of constraints. As the temperature is increased, the cooperative interactions which produce the constraint are reduced and more single-stranded regions become free to form single-stranded stacked regions. Since the stabilization energy of these stacks increases in the sequence U<C<A<G (7), at a given temperature we expect single-stranded stacked regions to contain more G than A and more A than U or C. Since the single-stranded regions of 16S RNA contain about 13.5% of the G residues and 12.5% of A residues (4), many of

these bases as well as those in the base-paired regions may take part in single-stranded stacks at 85°C. Evidence for such highly strained regions in RNA has also been provided by studies of the rate of RNase catalyzed hydrolysis of the RNA which contains a small number of sites with rate constants which are much larger than those observed for an unconstrained polynucleotide such as poly U (3, 8).

An alternative explanation of the present findings is that the magnitude and direction of perturbations in Raman intensities are determined significantly by base sequences. For example, the G lines in RNA may be genuinely hyperchromic. This seems unlikely however in view of the nature of the ultraviolet hypochromic effect in nucleic acids. We are currently studying model systems, including poly G, poly A · poly U and poly G · poly C to resolve this question conclusively. Attempts to eliminate the severe background scattering in Raman spectra of 23S RNA are also being made.

REFERENCES

1. G. J. Thomas, Jr., in Physical Techniques in Biological Research, G. Oster, Ed., Volume 1A, Optical Techniques, Second Edition. (Academic Press, N. Y., 1971).
2. G. J. Thomas, Jr., Biochim. Biophys. Acta 213, 417 (1970).
3. K. A. Hartman, J. Amaya and E. M. Schachter, Science 170, 171 (1970).
4. K. A. Hartman and G. J. Thomas, Jr., Science 170, 740 (1970).
5. G. J. Thomas, Jr. and J. R. Barylski, Appl. Spectry. 24, 463 (1970).
6. E. W. Small and W. L. Peticolas, Biopolymers 10, 69 (1971).
7. G. Felsenfeld and H. T. Miles, Ann. Rev. Biochem. 36, 407 (1967).
8. K. A. Hartman and J. Amaya, in preparation.