Raman Spectra of Ten Aqueous Transfer RNAs and 5S RNA. Conformational Comparison with Yeast Phenylalanine Transfer RNA†

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ABSTRACT: Eleven native transfer RNAs have been prepared so as to maintain their Mg^{2+} content. Their aqueous Raman spectra show a high, relatively constant amount of order in the ribophosphate backbone, as indicated by the ratio 1.73 ± 0.05 for I_{814}/I_{1100} in all samples. Variation in the effectiveness of stacking of guanine and adenine bases is seen, though most of the transfer RNAs studied have a comparable degree of stacking to that found in phenylalanine transfer RNA from yeast, whose tertiary structure has been determined by X-ray crystallography. The spectrum of *Escherichia coli* 5S RNA indicates that the stacking efficiency of the guanine bases is much higher in 5S RNA than in yeast phenylalanine transfer RNA, while that of the adenine bases is lower.

The structural features which enable tRNAs to be simultaneously diverse and similar are of considerable interest and importance to the understanding of protein biosynthesis (Rich and RajBhandary, 1976). All tRNAs pass through the ribosomal machinery (except for the initiator tRNA), in which they have the similar role of converting the genetic information from the messenger RNA into the amino acid sequence. Similarities also appear in the primary sequences of the tRNAs, which can all be arranged into the well-known cloverleaf form (Barrell and Clark, 1974). The commonality of tRNA three-dimensional structures is suggested by the structure of yeast $tRNA^{Phe}$, in which nucleotide bases common to all sequences are used in maintaining the fold of the molecule (Kim et al., 1974a; Robertus et al., 1974). In addition, it has been proposed that the structure of yeast $tRNA^{Phe}$ can be used as a model for understanding the three-dimensional folding of all tRNAs.

On the other hand, each tRNA has to be specifically recognized and charged by its cognate aminoacyl-tRNA synthetase, which implies that each tRNA has certain unique structural characteristics, however subtly they may vary from one tRNA to another. Obviously, the anticodon triad is one such characteristic and doubtless others will come to be recognized. However, the fact that aminoacyl-tRNA synthetases can operate to charge tRNAs with an incorrect amino acid indicates that the critical differences between them are not great (Giegé et al., 1974).

Detailed knowledge of the tertiary structure of tRNA has been derived mainly from X-ray diffraction studies of single crystals. However, despite the efforts of several laboratories (Rich and RajBhandary, 1976; Giegé et al., 1977), very few tRNAs have been obtained in crystalline forms that give suitable diffraction patterns. Thus, the investigation of the structures of tRNAs by other physical techniques such as laser Raman spectroscopy assumes a special importance. Previous Raman studies of tRNAs, including *E. coli* tRNAs in aqueous solution (Thomas et al., 1973a), yeast $tRNA^{Phe}$ in the crystal and aqueous solution (Chen et al., 1975), and others (Hartman et al., 1973), have shown the sensitivity of this technique to conformational changes in tRNAs by virtue of the relationship between intensities of Raman lines characteristic of the vibrations of various bases (or of the phosphodiester backbone) and the stacking of the bases (or the conformation of the backbone). In particular, the identity of the spectrum of yeast $tRNA^{Phe}$ in the crystal and in solution provides important information about the identity of conformation in these two media.

The present paper extends these earlier studies with particular regard to a comparison of the similarities and individual differences of a number of tRNAs. In addition, spectra have been obtained and analyzed for *E. coli* 5S RNA, a relatively small ribosomal nucleic acid (mol wt ~ 40 000). While several structural models for 5S RNA have been proposed (Erdmann, 1976), little is known at present about the actual stacking of its bases. Thus, it is also of interest to compare its structure with that of yeast $tRNA^{Phe}$.

Experimental Section

Unfractionated tRNA and purified $tRNA^{Phe}$ from brewer's yeast and $tRNA^{Glu}$ from *E. coli* were purchased from Boehringer-Mannheim. Other purified brewer's yeast tRNAs were prepared from partially purified countercurrent distribution fractions (Dirheimer and Ebel, 1967). Complete purification was carried out by column chromatography (Gillam et al., 1967) on BD cellulose (from Schwarz/Mann) and/or by reverse-phase chromatography with the RPC 5 system (Pearson et al., 1971) according to methods described previously: $tRNA^{Ala}$ (Giegé et al., 1971), $tRNA^{Asp}$ (Keith et al., 1971), $tRNA_f^{Met}$ and $tRNA^{Gly}$ (Geigé and Kern, unpublished results), $tRNA^{Leu}$ (Kowalski et al., 1971; Chang et al., 1973), $tRNA^{Trp}$ (Keith et al., 1971), and $tRNA^{Tyr}$ and $tRNA^{Val}_2$ (Bonnet, 1975). *E. coli* $tRNA^{Val}_1$ was purified from bulk *E. coli* tRNA (Schwarz/Mann). The major $tRNA^{Val}_1$ species was separated from the minor $tRNA^{Val}_2$ on a BD-cellulose

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TABLE I: Relative Intensities of Principal Raman Lines in 11 tRNAs and 5S RNA.[a]

freq (cm^{-1})	origin	Y Phe[b]	Y Ala	Y Asp	Y fMet	Y Gly	Y Leu	Y Trp	Y Tyr	Y Val	E Val	E Glu	5S RNA
670	G	0.44	0.57	0.57	0.43	0.45	0.38	0.39	0.47	0.37	0.52	0.56	0.69
725	A	0.69	0.62	0.62	0.60	0.54	0.69	0.68	0.77	0.56	0.62	0.55	0.66
785	C,U	2.16	2.21	2.30	2.39	2.08	2.07	2.33	2.40	2.28	2.39	2.44	2.44
814	-OPO-	1.74	1.68	1.79	1.75	1.75	1.78	1.69	1.75	1.69	1.74	1.77	1.54
1100	PO_2^-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1242	U,C,A[c]	1.37	1.36	1.39	1.54	1.38	1.37	1.38	1.43	1.42	1.63	1.63	1.57
1300	A,C	1.12	1.05	1.05	1.22	1.10	1.26	1.00	1.21	1.18	1.19	1.30	1.19
1321	G	1.34	1.34	1.42	1.48	1.34	1.24	1.14	1.48	1.28	1.70	1.35	1.57
1338	A	1.54	1.03	1.13	1.33	1.25	1.56	1.36	1.55	1.43	1.62	1.20	1.59
1375	A,G	1.07	0.96	0.96	1.05	0.98	1.01	0.94	1.01	0.99	0.94	0.94	1.11
1485	A,G	1.90	1.94	2.19	2.08	1.89	1.84	1.75	2.13	1.70	2.26	2.06	2.67
1575	A,G	1.40	1.22	1.36	1.45	1.27	1.44	1.25	1.56	1.28	1.62	1.46	1.67
	composit.[a]												
	A	18	8	13	16	13	21	17	17	14	15	14	23
	U	14	13	16	8	15	18	16	10	14	11	11	20
	G	22	27	24	27	23	23	20	23	21	24	22	41
	C	18	22	19	22	20	20	18	21	21	23	27	36
	other	4	6	3	2	3	3	3	7	6	3	2	0
	total	76	76	75	75	74	85	74	78	76	76	76	120

[a] All solutions were 3-4% by weight. Intensities are peak intensities near the indicated frequency relative to the PO_2^- line at 1100 cm^{-1}. For shoulders, the relative ordinate value at the indicated frequency is given. All values are averages of at least four spectra. Y = yeast tRNA, E = *E. coli* tRNA. For composition, minor bases are grouped with parent major base. Pseudo-U is included in U but dihydro-U with "other" because its Raman frequencies do not coincide with those of U (Thomas et al., 1973). [b] Intensities for yeast $tRNA^{Phe}$ were taken from Chen et al. (1975). [c] The peak near 1242 cm^{-1} is a superposition of lines near 1234 due to U and near 1251 due to A and C.

column (Gillam et al., 1967) and purified by the derivatization method of Gillam et al. (1968).

The purity of the tRNAs was checked before and after the Raman experiments by polyacrylamide gel electrophoresis (Gould, 1967), and the activity of the various species was assayed by aminoacylation in the presence of crude baker's yeast or *E. coli* extracts (Giegé et al., 1972). All tRNAs tested had activities higher than 1500 pmol of amino acid per unit of optical density at 260 nm.

All tRNAs were dialyzed at 4 °C against the same solutions: twice against 40 mM sodium cacodylate buffer (pH 6.0) containing 2 mM $MgCl_2$ and twice against 2 mM $MgCl_2$. Solutions of 3-4% (w/w) and the same ionic strength were prepared for Raman measurement. The only sample whose ionic composition requires special comment is that of yeast $tRNA^{Leu}$, in which 10 mM $MgCl_2$ and 10 mM cacodylate ion as buffer (pH 7) are present.

Purified *E. coli* K12 5S RNA was a gift from BioLabs. It was dialyzed at 4 °C against 2 mM $MgCl_2$ for 12 h and then lyophilized. An aqueous solution of approximately 5% (w/w) was used for the Raman studies.

The Raman instrumentation (Spex Ramalog Model 4 and Coherent Radiation 52G Ar^+ laser) and sample handling for the Raman experiments have been described in detail elsewhere (Chen et al., 1975). The intensity measurements for all resolved lines are peak heights above background measured with respect to the PO_2^- line at 1100 cm^{-1} at a spectral slit width of 8 cm^{-1}. The peak height at 1100 cm^{-1} was determined from a baseline drawn tangent to the spectrum at approximately 1065 and 1130 cm^{-1}. Normalization to the 1100 line has been found a reliable procedure by several authors (Thomas et al., 1972, 1973a; Brown et al., 1972; Thomas and Hartman, 1973; Chen et al., 1975). For the doublet at 785 and 814 cm^{-1} the intensity overlap at these wavenumbers is small, as measured from a baseline tangent at approximately 740 and

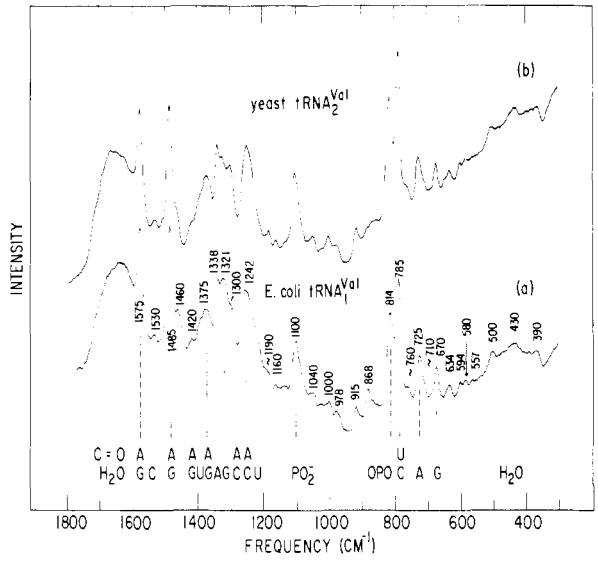

FIGURE 1: Raman spectra of 3% aqueous tRNAs containing 2 mM $MgCl_2$: (a) *E. coli* $tRNA^{Val}_1$; (b) yeast $tRNA^{Val}_2$. The origins of the major Raman lines are indicated by: A, adenine; U, uracil; G, guanine; C, cytosine; OPO, phosphodiester chain mode; PO_2^-, ionized phosphate. When two bases contribute to the intensity of a given line, the symbol of the major contributor is placed above the other.

840 cm^{-1} with an assumption of Gaussian line shapes for the individual components; hence, the peak heights for the doublet are valid. In the region 1200-1430 cm^{-1} the overlapping is far more serious, and quantitative determination of line intensities from a given individual base is difficult. We have chosen to report simply the measured peak heights above a baseline drawn between about 1170 and 1440 cm^{-1}. The relative intensities reported herein are the average of four or more individual spectra. Their reproducibility is ±3%.

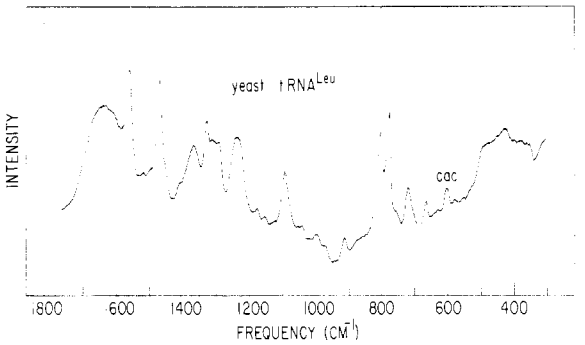

FIGURE 2: Raman spectrum of 3% aqueous yeast tRNA^{Leu} containing 10 mM $MgCl_2$. The line at 610 cm^{-1} labeled "cac" is due to 10 mM cacodylate ion buffer.

Results and Discussion

The Raman intensities of 11 tRNAs and 5S RNA are tabulated in Table I together with the base composition of each. For illustrative purposes, Figure 1 shows the original spectra of tRNA^{Val} from *E. coli* and from brewer's yeast and Figure 2 the spectrum of yeast tRNA^{Leu}. The first two spectra are included to enable comparison of tRNAs for the same amino acid from two different organisms and the third is shown as an example of a tRNA having a large extra loop (Kowalski et al., 1971; Chang et al., 1973).[1] The spectra also are useful as a guide to the appearance of the remaining eight spectra, which can be inferred from the intensities in Table I. A comparative discussion of these spectra can conveniently be given in terms of their similarities and differences.

Similarities in the Raman Spectra of Various tRNAs. The intense line at 814 cm^{-1} arising from the phosphodiester group has been widely recognized as a measure of the regularity of the ribophosphate backbone in polynucleotides (Yu, 1969; Thomas, 1970; Brown et al., 1972; Thomas and Hartman, 1973; Brown and Peticolas, 1975), since this frequency is strongly dependent on the geometry of the C-O-P-O-C linkage (Brown and Peticolas, 1975; Forrest and Lord, 1977). Hence, it is an important observation that the intensity of this line in all the native tRNAs that we have studied is approximately 1.7 times that of the line at 1100 cm^{-1} due to the symmetric stretching mode of the PO_2^- group. The latter line has been used as the standard of intensity for numbers in Table I because experience (Chen et al., 1975; Brown et al., 1972; Thomas and Hartman, 1973; Thomas et al., 1972; 1973a) has shown it to be quite insensitive in integrated intensity to structural changes and relatively insensitive in peak intensity provided the ionic strength does not depart seriously from that of the native condition. If the line at 1100 cm^{-1} shows unusual half width or asymmetry, the presumption is that the integrated intensity must be used. For example, in Figure 1a, the 1100 line is asymmetric on the low-frequency side, and the peak intensity ratio I_{814}/I_{1100} has the unusually high value of 1.79 for tRNA^{Val}. When the integrated areas are used, the ratio is reduced to 1.74.

The values of I_{814}/I_{1100} in Table I are substantially larger than those found by earlier workers for a number of tRNAs. These include fMet, Val and Phe_2 from *E. coli* (Thomas et al., 1972), Glu and Arg from *E. coli* (Thomas et al., 1973a), and unfractionated tRNA from yeast (Small et al., 1972; Thomas et al., 1973b), for which the reported ratios are close to 1.4. As we have commented earlier (Chen et al., 1975), it is not always clear whether to attribute this evidence of some backbone disorder to depletion of Mg^{2+} ion or to other causes. The fact that the ten additional tRNAs studied here, which were prepared in such a way that the native Mg^{2+} content was not changed, yield very nearly the same high value of this ratio (Table I) shows that the regularity in the ribophosphate backbone structure is the same in all of them. One is accordingly encouraged to consider their backbone structures to be the same as that found for yeast tRNA^{Phe} (Kim et al., 1974a; Robertus et al., 1974).

The intensities of the Raman lines due to the various bases in a given spectrum depend of course on the relative abundances of the bases in that tRNA, as well as on their stacking. However, the frequencies of these lines are characteristic of the individual bases (Lord and Thomas, 1967), as indicated in Table I. Thus, one expects, and finds, a qualitative similarity among the various spectra, since the base compositions of tRNAs vary within rather narrow limits. The exploitation of the differences in the spectra of the different tRNAs therefore depends on a quantitative interpretation of intensities of lines whose qualitative meaning has been well established. This is also true of the differences observed within the spectrum of a given tRNA when the parameters that affect its structure (pH, temperature, concentrations of various ions, etc.) are varied.

Differences in the Spectra of Various tRNAs. As just mentioned, the base composition of a given tRNA determines in the first instance its quantitative Raman spectrum. However, the contributions of the individual bases to the region below 800 cm^{-1} (Table I) are less complicated by overlapping than those in the range 1200–1600 cm^{-1}. Hence, the lines at 670 cm^{-1} due to G and 725 cm^{-1} due to A (and to some extent that at 785 cm^{-1} due to both C and U) are the most useful in measuring those characteristics of the tRNA structure that relate to these bases. As an example of this, we may compare the intensities of the two G and A lines in native tRNA^{Leu} and tRNA^{Phe} from yeast. If one assumes that the unit contribution of each base to the intensity of its Raman lines is the same in both tRNAs and corrects for the differences in the number of G and A bases and the number of phosphate residues (which of course influences the peak intensity of the reference line at 1100 cm^{-1}), one calculates an expected intensity I_{calcd} in tRNA^{Leu} compared to that observed in tRNA^{Phe} as follows:

$$I_{calcd}(tRNA^{Leu}) = I_{obsd}(tRNA^{Phe})\left[\frac{N_B(Leu) \times N_R(Phe)}{N_B(Phe) \times N_R(Leu)}\right]$$

where N_B is the number of bases (G or A) in Leu and Phe and N_R is the total number of nucleotide residues in each tRNA. Thus, one calculates that the intensity expected for the G line at 670 cm^{-1} in tRNA^{Leu} from the intensity of the same line in tRNA^{Phe} should be 0.41 (observed 0.38). For the A line at 625 cm^{-1} the same calculation yields 0.72 (observed 0.69).

The agreement of expected and observed values is within experimental error for the A line and possibly so for the G line. This agreement is an important piece of information, because the effect of the stacking of the G and A bases on these intensities is considerable. In the spectrum of yeast tRNA^{Phe} (Chen et al., 1975), the 670-cm^{-1} line *decreases* by a factor of 3 when the G bases go from the native (all-stacked) configuration (Kim et al., 1974a; Ladner et al., 1975) to the unstacked form, while the 725-cm^{-1} line *increases* by 12% as the partially stacked A residues in the native form are unstacked. Hence,

[1] In Figure 2 there is a line of medium intensity at 610 cm^{-1} that does not occur in the other spectra. It arises from the cacodylate ion present at about 10 mM as a buffer in the solution of yeast tRNA^{Leu}. This addition to the ionic environment appears from the spectrum to have made no change in the backbone structure of tRNA^{Leu}, although it corresponds to about ten cacodylate ions per molecule of tRNA^{Leu}.

TABLE II: Calculated and Observed Relative Intensities of G and A Lines.[a]

tRNA	line at 670 cm^{-1} (G)		line at 725 cm^{-1} (A)	
	calcd	obsd	calcd	obsd
Y Phe	(0.44)	0.44	(0.69)	0.69
Y Ala	0.54	0.57	0.31	0.62
Y Asp	0.49	0.57	0.51	0.62
Y fMet	0.55	0.43	0.62	0.60
Y Gly	0.47	0.45	0.52	0.54
Y Leu	0.41	0.38	0.72	0.69
Y Trp	0.41	0.39	0.67	0.68
Y Tyr	0.42	0.47	0.64	0.77
Y Val	0.42	0.37	0.54	0.56
E Val	0.48	0.52	0.58	0.62
E Glu	0.44	0.56	0.54	0.55
5S RNA	0.52	0.69	0.56	0.66

[a] For G, if observed is *larger* than calculated, more stacking than in $tRNA^{Phe}$; for A, if observed is *smaller* than calculated, more stacking than in $tRNA^{Phe}$.

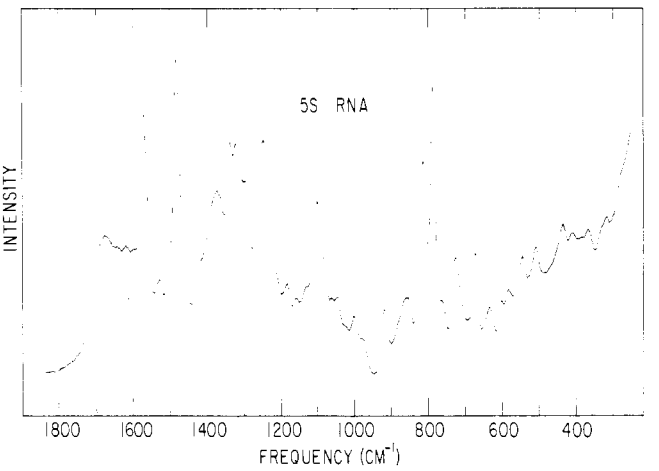

FIGURE 3: Raman spectrum of 5% aqueous *E. coli* 5S RNA containing 2 mM $MgCl_2$.

the agreement between calculated and observed ratios of these two lines in native $tRNA^{Leu}$ and $tRNA^{Phe}$ indicates that the *fraction* of stacked A and G in the former is about the same as in the latter.

The above calculation has been made for all the tRNAs studied from the data of Table I, and the results are given in Table II. Comparison of the observed with the calculated G and A line intensities gives an interesting view of base stacking in the various tRNAs. It must be kept in mind that when the observed G line intensity is *larger* than calculated, the G bases for that tRNA are more effectively stacked on the average than for $tRNA^{Phe}$; for the A line, the opposite is true of the A bases. The largest differences for the 690-cm^{-1} line are found for $tRNA^{Asp}$, $tRNA_f^{Met}$, and $tRNA^{Tyr}$ from yeast and $tRNA^{Glu}$ from *E. coli*. All of these, except the fMet, show more effective G-base stacking than $tRNA^{Phe}$, and all have a higher fraction of paired G bases in the stems, where the stacking is presumably more regular. For example, in $tRNA^{Phe}$ there are 22 G's, of which 13 are stacked in the stems and eight in the tertiary structure (one, G_{20}, is apparently not stacked at all) (Kim et al., 1974a; Ladner et al., 1975), whereas in yeast $tRNA^{Asp}$ there are 24 G's, of which 17 are in the stems and seven outside (Barrell and Clark, 1974). The $tRNA_f^{Met}$, however, shows *less* effective G stacking. The spectroscopic result needs to be checked against other fMet samples, but $tRNA_f^{Met}$ appears to have a more open structure so far as its G stacking is concerned.

The 725-cm^{-1} line shows the largest differences for $tRNA^{Ala}$, $tRNA^{Asp}$, and $tRNA^{Tyr}$ from yeast, with the stacking of the A bases *less* effective than in the $tRNA^{Phe}$. In all three the fraction of paired A bases in the stems is very much less than in $tRNA^{Phe}$, and substantially less effective stacking is expected. For all the other tRNAs studied here, this fraction is equal to or greater than that of $tRNA^{Phe}$ except for fMet and Glu. However, since the $tRNA_f^{Met}$ is anomalous in G stacking, it may well have a tertiary structure that depends for its stability, among other factors, on the effective stacking of its A bases, only three of which are paired in the stems. The fraction of A bases in the stems in Glu, 0.29 compared with 0.44 in Phe, might lead to somewhat less overall A stacking, but the agreement between observed and calculated intensities simply says that despite this the A bases are as effectively stacked in $tRNA^{Glu}$ as in $tRNA^{Phe}$.

The foregoing illustrates the usefulness of the two G and A lines for the comparison of the overall G and A stacking in different tRNAs. They are also valuable for following the changes in stacking in a given tRNA with temperature and other parameters. We have already shown (Chen et al., 1975) such an application for $tRNA^{Phe}$, and it would clearly be of interest to observe the quantitative changes of the intensities in Tables I and II as a function of these parameters. Unfortunately, the C + U line at 785 cm^{-1} is not so easy to exploit for these purposes, since it shows only the combined effect of the behavior of the two bases, and in addition overlaps the OPO line at 814 cm^{-1}, from which it must be separated for quantitative work.

The region 1200–1600 cm^{-1} contains strong lines due to ring vibrations of the individual bases (Lord and Thomas, 1967). The line intensities depend on the relative abundances of the bases and on their stacking, but as Figures 1 and 2 illustrate the lines are rather badly overlapped in the range 1200–1450 cm^{-1} and the contour, which is a kind of "fingerprint" of a given tRNA, must be quantitatively dissected into its individual components before the information can be used to follow the behavior of the several bases.

All the lines in this region show hypochromism. This is rather strikingly illustrated, for example, by the line due to U at 1234 cm^{-1} when $tRNA^{Phe}$ is thermally denatured (Figure 2c of Chen et al., 1975). However, the close overlapping of this line with the pair due to C and A at 1251 cm^{-1} (compare the unresolved complex at 1242 cm^{-1} in Figures 1 and 2) makes unreliable the quantitative determination of U-base stacking by means of it. Similar comments can be made about the contour between 1300 and 1350 cm^{-1}, where four lines due to C, A, G, and A are superimposed. This contour alters if the temperature or Mg^{2+} concentration is suitably changed, as has been shown by Thomas, Chen, and Hartman (1973a) for $tRNA^{Glu}$ from *E. coli* and by the present authors (1975) for yeast $tRNA^{Phe}$.

Comparison of the Spectra of 5S RNA and tRNAs. Figure 3 shows the Raman spectrum of 5% aqueous 5S RNA from *E. coli* K12. The peak intensities, averaged from five spectra, of the principal lines relative to I_{1100} are included in Table I. The regularity of the ribophosphate backbone as indicated by the value of 1.54 for I_{814}/I_{1100} is noticeably less than that observed for all the native tRNAs examined. This is somewhat surprising, since the number of bases paired in the stems is indicated both by the ultraviolet hypochromism (Boedtker and Kelling, 1967; Monier, 1974) and by the Raman data for G and A separately to be relatively larger in 5S RNA than in

$tRNA^{Phe}$. The expected intensity of the 670-cm^{-1} line, 0.52 (calculated as for Table II), is decidedly less than that observed, 0.69, which implies that the G-bases are more effectively stacked than in $tRNA^{Phe}$. Since about 59% of the G bases are paired up (Kim et al., 1974a; Robertus et al., 1974) in the stems in $tRNA^{Phe}$, it is concluded that the fraction of paired G's in the stems of 5S RNA must be much larger. In the review of Erdmann (1976), a number of proposed structures for 5S RNA are described, some of which, such as that of Boedtker and Kelling (1967), have fractions of paired G's in the vicinity of 0.70, a value quite consistent with the Raman data. Interestingly the same model has only 35% of the A bases paired in the stems, lower than the 44% found in $tRNA^{Phe}$. This shows clearly in the Raman line at 725 cm^{-1}, where the calculated intensity, 0.56, is well below the observed, 0.66, which indicates less stacking of A in 5S RNA than in $tRNA^{Phe}$.

The low-frequency region (300–600 cm^{-1}) contains several clearcut lines of moderate intensity. The origins of these lines are not obvious, but they apparently do not arise from vibrations of the individual bases, since their positions do not correspond with any of the base frequencies reported by Lord and Thomas (1967).

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