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Infrared Spectra of a Few Transfer Ribonucleic Acids

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Infrared absorptions have been observed in the 1800—700 cm^{-1} region of four purified amino-acid transfer ribonucleic acids (tRNA's): alanine tRNA and valine-I tRNA from *Torulopsis utilis* (torula yeast) and methionine tRNA and tyrosine tRNA from *Escherichia coli* in their deuterated film. Spectral features in the 1550—1750 cm^{-1} region of the valine, methionine, and tyrosine tRNA's indicate that each of these tRNA's has an amount of base-pairs, which is in accord with the "clover leaf" structure previously proposed. The relative amounts of the paired and unpaired cytosine residues are estimated on the assumption that the intensity ratio of the 1523 and 1500 cm^{-1} bands is 0.18 for the paired cytosine residue and 0.84 for the unpaired cytosine residue. The results are also compatible with the "clover leaf" structure.

This is a preliminary report of our infrared absorption measurements of four purified amino-acid transfer ribonucleic acids (tRNA's). Our aim of such measurements is to obtain an information of the secondary structure of a tRNA and of its change caused by a change of the environment. Here we describe only the results obtained of the deuterated films of these tRNA's, from which a piece of the desired information has been obtained.

Abbreviations used in this paper are AMP = adenosine-5′-monophosphate, UMP = uridine-5′-monophosphate, GMP = guanosine-5′-monophosphate, CMP = cytidine-5′-monophosphate, A = unpaired adenosine, U = unpaired uridine, G = unpaired guanosine, C = unpaired cytidine, AU = adenine-uracil base pair, and GC = guanine-cytosine base pair, Ψ = pseudouridine, DiHU = 5,6-dihydrouridine.

Experimental

Four tRNA samples were used: (1) alanine-specific tRNA from *Torulopsis utilis* (torula yeast), (2) one of the two valine-specific tRNA's (named "valine-I tRNA") from *T. utilis*, (3) methionine-specific tRNA from *Escherichia coli*, and (4) tyrosine-specific tRNA from *E. coli*. The former two were prepared as has been described by Takemura *et al.*[1,2] and the latter two as has been described by Nishimura *et al.*[3] The first two samples were precipitated by ethanol, washed by ether, and dried in vacuum. Each of the last two samples (about 1 mg) was brought into a concentrated aqueous solution. This was dialysed against a 0.01 M $MgCl_2$ solution to remove polyatomic anions and then lyophilized. A film of each sample was prepared on a AgCl plate. The control of the humidity of the air over the sample film and deuteration of the sample were made by the method described by Sutherland and Tsuboi.[4]

Results and Interpretation

1) General Feature. In Fig. 1, whole the spectrum of the 700—1750 cm^{-1} region observed of alanine tRNA is illustrated. Here, the recorded transmission-wavenumber curve is converted into an absorbance-wave number curve, because one of our aims is to conduct a quantitative analysis (as to the amounts of nucleotide residues located at different intramolecular environments).

In many respects, the spectral features of the four tRNA's are similar to one another, to that of double-helical RNA from rice dwarf virus,[5] and to that of ribosomal RNA from rat liver.[6] All of the four tRNA's (deuterated) give strong absorptions in the 1070—1100 cm^{-1} region, 1220—1260 cm^{-1} region, and 1640—1700 cm^{-1} region. These are attributable respectively to the PO_2^- symmetric

1) S. Takemura, M. Miyazaki, M. Kawata, T. Mizutani, S. Hashimoto and M. Murakami, 7th Intern. Congr. Biochem., Symp. I-5, 1967, Tokyo.

2) M. Kawata, M. Miyazaki and S. Takemura, *J. Biochemistry (Tokyo)*, **62**, 287 (1967).

3) S. Nishimura, F. Harada, U. Narusima and T. Seno, *Biochim. Biophys. Acta*, **142**, 133 (1967).

4) G. B. B. M. Sutherland and M. Tsuboi, *Proc. Roy. Soc. (London)*, **A239**, 446 (1957).

5) T. Sato, Y. Kyogoku, S. Higuchi, Y. Mitsui, Y. Iitaka, M. Tsuboi and K. Miura, *J. Mol. Biol.*, **16**, 180 (1966).

6) Y. Kyogoku, Doctor Thesis, p. 28, the University of Tokyo, 1963.

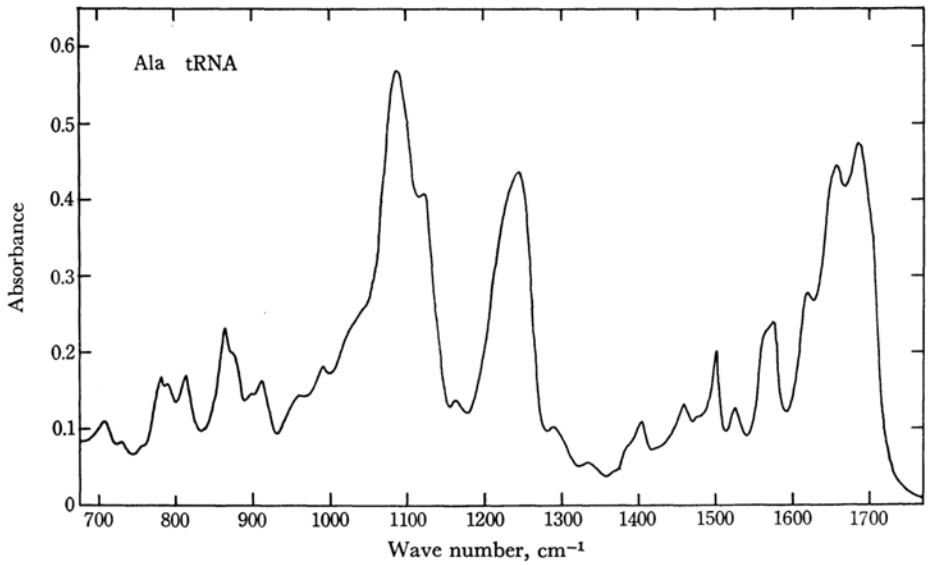

Fig. 1. Infrared absorption curve (in the 700—1750 cm^{-1} region) of a deuterated film of alanine tRNA from *Torulopsis utilis* placed in the atmosphere of 92% relative humidity (D_2O vapor).

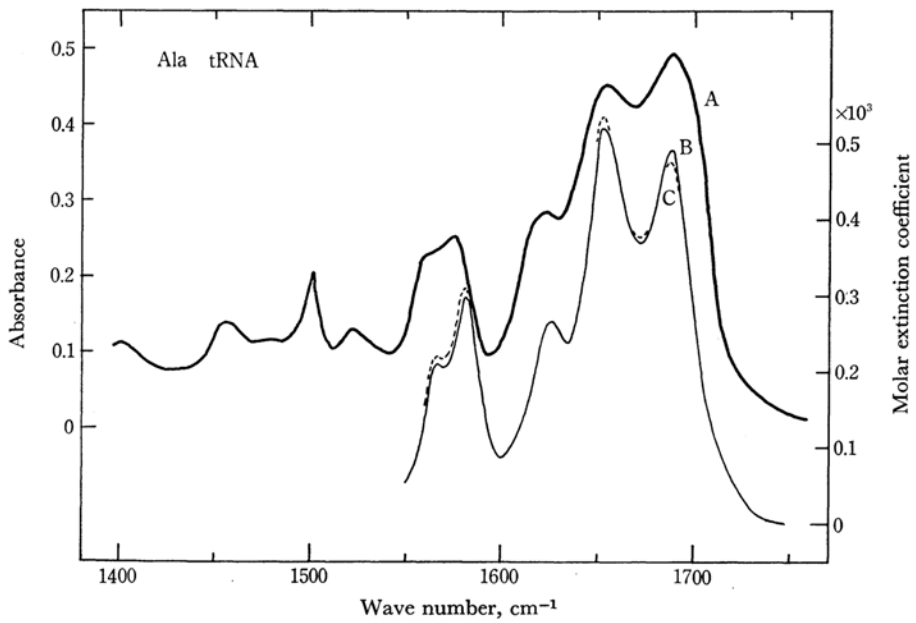

Fig. 2. A: Infrared absorption curve observed (in the 1400—1750 cm^{-1} region) of a deuterated film of alanine tRNA from *Torulopsis utilis* placed in the atmosphere of 92% relative humidity (D_2O vapor).

B: Calculated curve (1550—1750 cm^{-1}) for 0.060 AU + 0.508 GC + 0.090 AMP + 0.134 UMP + 0.119 GMP + 0.089 CMP (corresponding to 2 AU + 17 GC + 6A + 9U + 8G + 6C).

C: Calculated curve (1550—1750 cm^{-1}) for 0.060 AU + 0.478 GC + 0.090 AMP + 0.134 UMP + 0.134 GMP + 0.104 CMP (Corresponding to 2AU + 16GC + 6A + 9U + 9G + 7C).

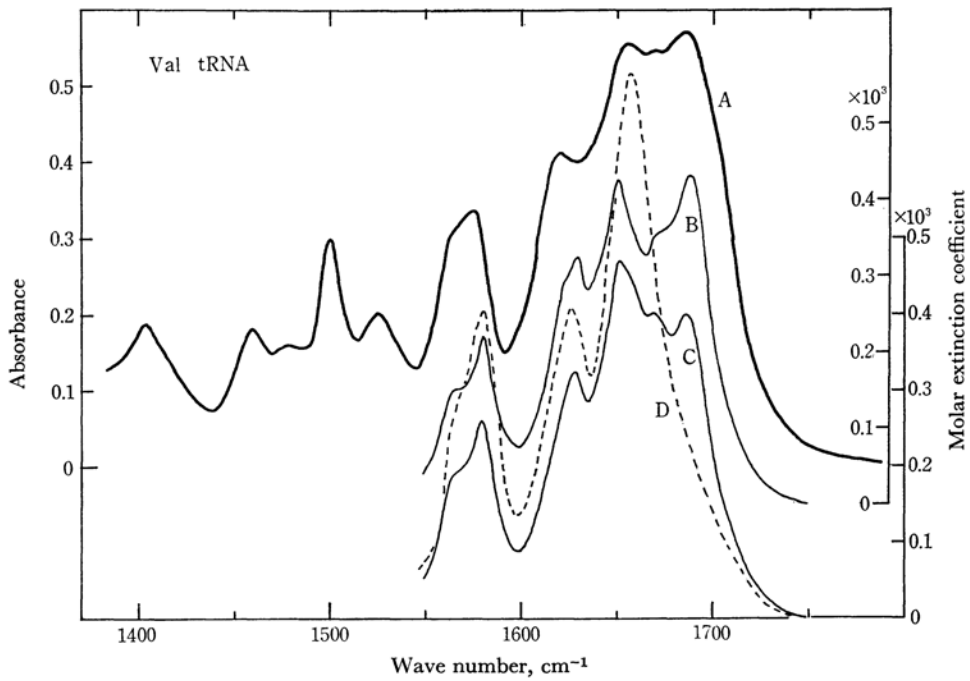

Fig. 3. A: Infrared absorption curve observed (in the 1400—1750 cm^{-1} region) of a deuterated film of valine-I tRNA from *Torulopsis utilis* placed in the atmosphere of 92% relative humidity (D_2O vapor). B: Calculated curve (1550—1750 cm^{-1}) for 0.219 AU + 0.406 GC + 0.125 AMP + 0.063 UMP + 0.063 GMP + 0.125 CMP (corresponding to 7 AU + 13 GC + 8A + 4U + 4G + 8C). C: Calculated curve (1550—1750 cm^{-1}) for 0.187 AU + 0.312 GC + 0.125 AMP + 0.094 UMP + 0.125 GMP + 0.156 CMP (corresponding to 6AU + 10 GC + 8A + 6U + 8G + 10C). D: Calculated curve (1550—1750 cm^{-1}) for 0.222 AMP + 0.175 UMP + 0.286 GMP + 0.318 CMP (corresponding to 14A + 11U + 18G + 20C).

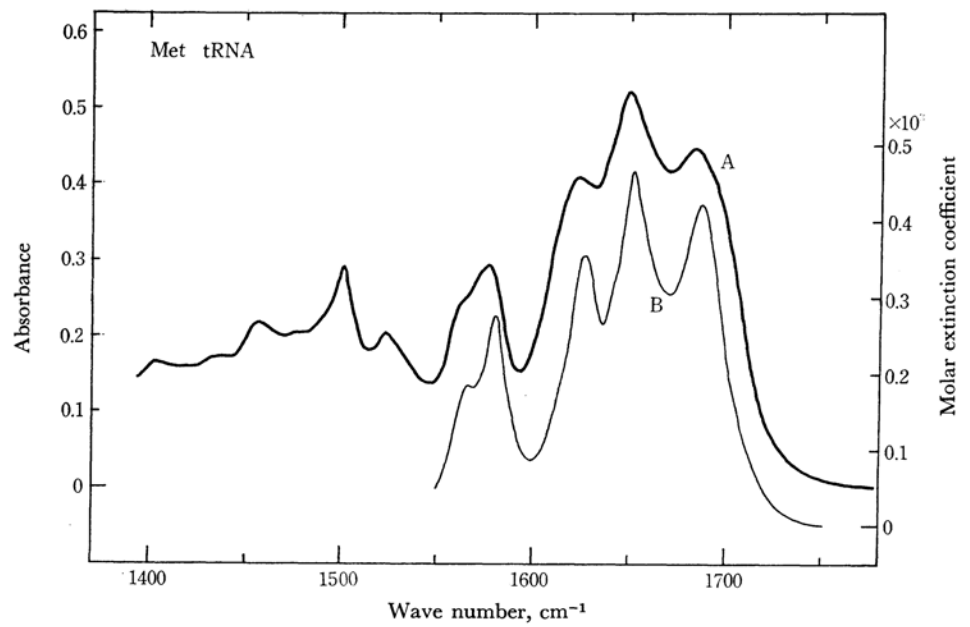

Fig. 4. A: Infrared absorption curve (in the 1400—1750 cm^{-1} region) of a deuterated film of methionine tRNA placed in the atmosphere of 75% relative humidity (D_2O vapor). B: Calculated curve (1550—1750 cm^{-1}) for 0.057 AU + 0.486 GC + 0.186 AMP + 0.071 UMP + 0.086 GMP + 0.114 CMP (corresponding to 2 AU + 17 GC + 13A + 5U + 6G + 8C).

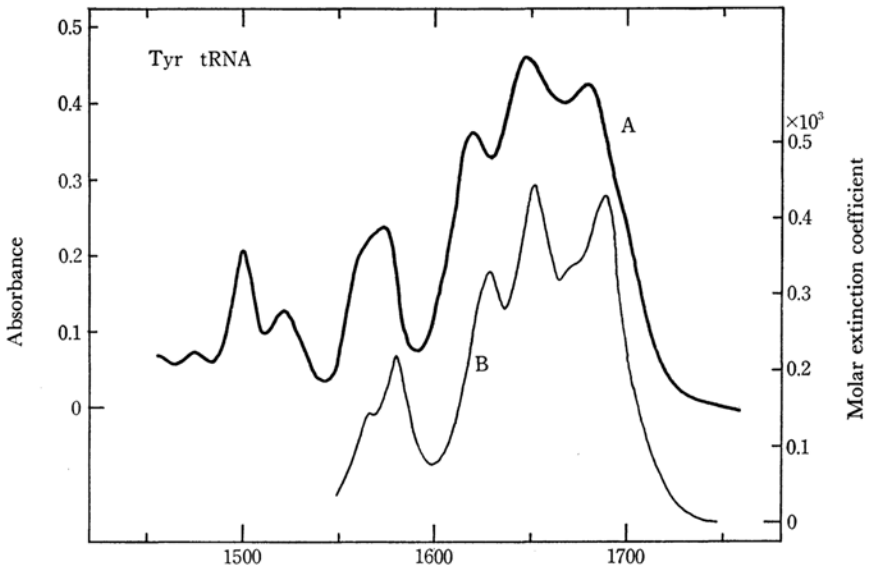

Fig. 5. A: Infrared absorption curve (in the 1450—1750 cm^{-1} region) of a deuterated film of methionine tRNA placed in the atmosphere of 75% relative humidity (D_2O vapor).

B: Calculated curve (1550—1750 cm^{-1}) for 0.177 AU + 0.430 GC + 0.139 AMP + 0.076 UMP + 0.051 GMP + 0.126 CMP (corresponding to 7AU + 17GC + 11A + 6U + 4G + 10C).

stretching, PO_2^- antisymmetric stretching + D_2O deformation, and in-plane stretching vibrations of the base residues.[4] There are moderately strong absorption bands at 1575 and 1500 cm^{-1} assignable respectively to the guanine and cytosine residues.[7,7a] The absorption bands at 1125 and 810 cm^{-1} are characteristic of RNA and are assigned to $\begin{matrix}C\\C\end{matrix}\rangle$C-O degenerate and symmetric stretching vibrations around the 2′C in the ribose residue.[8]

The details of the spectrum in the 1400—1750 cm^{-1} and 760—840 cm^{-1} regions, on the other hand, are somewhat different for different RNA's. The absorption curves of the four tRNA's in these spectral regions are shown in Figs. 2—6.

2) The 1550—1750 cm^{-1} Region. Recently, Thomas[9] described a method for evaluating of the fractions of AU and GC base pairs contributing to the secondary structure of a given RNA from its infrared absorption spectrum in the 1550—1750 cm^{-1} region. For such an evaluation, he presented a catalog of infrared absorption spectra of poly(A+U), poly(G+C), AMP, UMP, GMP, and CMP in their D_2O solutions. We have been attempting similar base analyses, and what we observed of poly(A+U) and poly(G+C) in D_2O

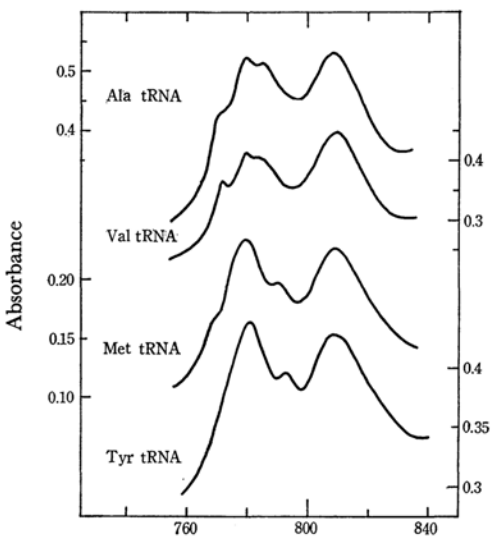

Fig. 6. Infrared absorption curves (760—840 cm^{-1} region) of deuterated films of alanine tRNA, valine-I tRNA, methionine tRNA, and tyrosine tRNA.

7) M. Tsuboi, K. Shuto and S. Higuchi, This Bulletin, **41**, 1821 (1968).

7a) H. Fritzsche, *Biopolymers,* **5**, 863 (1967).

8) M. Tsuboi, K. Matsuo, T. Shimanouchi and Y. Kyogoku, *Spectrochim. Acta,* **19**, 1617 (1963).

9) G. J. Thomas, Jr., *Biopolymers,* in press. We thank Dr. Thomas for kindly showing his results before publication.

are found to be in accord with what Thomas independently observed. We have not yet determined, however, the molar extinction coefficient for each point in the absorption curve, as Thomas did. In addition, we have been using the absorption curves of adenosine, uridine, guanosine, and cytidine in D_2O[10] which may be slightly less suitable than the curves of AMP, UMP, GMP, and CMP as the standard curves for evaluating the amounts of unpaired bases. Let us use, therefore, the standard curves presented by Thomas in our present infrared analyses.

The infrared base-analyses of tRNA's have an additional difficulty: we have no standard spectra of minor bases in tRNA. In the present preliminary analyses, we neglect their contribution. It is true that the total amount of the minor bases in each tRNA is not small at all. For example, valine-I tRNA molecule has altogether 12 minor base residues in the total 75 base residues. They consist, however, of smaller numbers of several different minor bases, and the contribution of each base is different from another and is rather small.

The observed absorption curve of our alanine tRNA (Fig. 2,A) indicates that some of the base residues in it are hydrogen-bonded (paired) and others are unpaired; the absorption peak at 1690 cm^{-1} is caused mainly by the AU and GC base pairs, whereas absorptions at 1655 and 1625 cm^{-1} are mainly attributable to the unpaired bases.[9,11] The determination of the chemical structure of alanine tRNA from torula yeast is not yet completed. For alanine tRNA from baker's yeast, however, the nucleotide sequence was determined by Holley *et al.*[12] On the basis of this sequence, Holley[13] suggested a "clover leaf" model,[13a] which contains 2 AU pairs, 17 GC pairs, and 6A, 9U, 8G, and 6C unpaired. On the basis of this base-pair composition, a synthetic spectrum in the 1550—1750 cm^{-1} region was calculated by the use of Thomas' standard curves. The result is given in Fig. 2, B. As another trial, a calculated curve (Fig. 2, C) was obtained for a "clover-leaf" model in which the so-called "DiHU arm.[14] has only three GC pairs instead of four (most of other "clover-leaf" models so far presented have only three base pairs in the "DiHU arm"). A comparison of the curves A, B, and C in Fig. 2 indicates that the structure of alanine tRNA of torula yeast is somewhat different from what was proposed of baker's yeast. It is suggested that the former has more base pairs or less unpaired bases than the latter, because the relative intensity of the 1690 cm^{-1} peak is found to be greater in the observed spectrum than that in the calculated spectra. In some respects, however, the alanine tRNA in question seems to be similar to the "clover-leaf" model of alanine tRNA of baker's yeast. For example, the relative intensity of the 1623 cm^{-1} peak is lower in the alanine tRNA in question than in the other three tRNA's examined (Figs. 3—5). This fact indicates that the number of unpaired A is relatively small in this tRNA. This is the case also in the "clover-leaf" model of alanine tRNA of baker's yeasts; there are only 6 A's, whereas in most of the "clover-leaf" models of other tRNA, there are more than 10 A's.

Valine-I tRNA should have 5AU, 12GC, 1AΨ, and 2GU base pairs and 8A, 4U, 4G, and 8C unpaired, if the "clover-leaf" model is adopted.[15] Because we do not yet know the standard curves for AΨ and GU pairs, let us assume for trial that there are 7AU and 13GC in obtaining the synthetic spectrum. The result is given in Fig. 3, B. Figure 3, C is a calculated spectrum for a structure which is obtained from the "clover-leaf" model by breaking the three base pairs (two GC and one GU) in the "DiHU arm"[14] and one GU pair in the "amino-acid arm."[14] (This may be the structure obtained in the first stage of the melting process of the "clover-structure). Figure 3, D is a calculated spectrum which is expected for the completely denatured form. Among these three calculated spectra (B, C, and D), spectrum B is in the best agreement with the observed spectrum (Fig. 3, A). Therefore, it may be concluded that valine-I tRNA now in question has base pairing contents which are almost equal to those in its "clover-leaf" model.

Similar examinations were made also for methionine and tyrosine tRNA's from *E. coli*. In each case, the calculated spectrum on the basis of the "clover-leaf" model[16,17] is in a good agreement with the observed spectrum (see Figs. 4 and 5). Both of these two tRNA's show relatively strong

10) M. Tsuboi, Y. Kyogoku and T. Shimanouchi, *Biochim. Biophys. Acta*, **55**, 1 (1962).

11) Y. Kyogoku, M. Tsuboi, T. Shimanouchi and I. Watanabe, *Nature*, **189**, 120 (1961).

12) R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick and A. Zamir, *Science*, **147**, 1462 (1965).

13) R. W. Holley, *Scientific American*, **214**, Feb. 30 (1966).

13 a) The "clover leaf" model is what was proposed by Holley[13] as one of possible models of the secondary structure of alanine-tRNA from baker's yeast. It has four arms named[14] "amino-acid arm," "T, Ψ arm," "anticodon arm," and "DiHU arm," which have seven, five, five and four base-pairs respectively. Nucleotide sequences determined later for a number of amino-acid specific tRNA molecules indicated that a similar model is reached when each tRNA molecule is folded so that the number of intramolecular Watson-Crick base-pairs is a maximum.

14) W. Fuller and A. Hodgson, *Nature*, **215**, 817 (1967).

15) S. Takemura, T. Mizutani and M. Miyazaki, *J. Biochemistry (Tokyo)*, in press.

16) S. K. Dube, K. A. Marcker, B. F. C. Clark and S. Cory, *Nature*, **218**, 232 (1968).

17) H. M. Goodman, J. Abelson, A. Landy, S. Brenner and J. D. Smith, *ibid*, **217**, 1019 (1968).

1650 and 1623 cm^{-1} peaks. This fact indicates that the relative amounts of the GC pair and unpaired A are great in these tRNA. These amounts are certainly great if their "clover-leaf" models are valid: 17GC and 13A in methionine tRNA and 17GC and 11A in tyrosine tRNA.

3) The 1400—1550 cm^{-1} Region. The moderately strong band at 1500 cm^{-1} is assigned to the cytosine residue.[7,7a] This band seems to appear irrespective of whether the cytosine residue is hydrogen-bonded or not. Another band attributable to the cytosine residue at 1523 cm^{-1}, on the other hand, is strong only when the residue is unpaired. The intensity ratio of the 1523 to 1500 cm^{-1} bands, $I(1523\ cm^{-1})/I(1500\ cm^{-1})$ is about 0.84 in completely heat denatured DNA in D_2O solution.[18] It has about the same value in cytidine and in CMP in D_2O solutions. While, the $I(1523\ cm^{-1})/I(1500\ cm^{-1})$ value is 0.18 in native double-helical DNA in D_2O. It has almost the same value for double-helical RNA.[5] Fortunately, there are almost no disturbing bands of other base residues in this spectral region, and therefore these two bands are useful in estimating the relative amounts of paired and unpaired cytosine residues. It is assumed that the mole ratio r of the paired cytosine *versus* total cytosine residues is given by

$$r = 1.27 - 1.52\ I(1523\ cm^{-1})/I(1500\ cm^{-1}) \quad (1)$$

so that $r=0$ when $I(1523\ cm^{-1})/I(1500\ cm^{-1}) = 0.84$, and $r=1$ when $I(1523\ cm^{-1})/I(1500\ cm^{-1}) = 0.18$. $I(1523\ cm^{-1})/I(1500\ cm^{-1})$ is found to be 0.30, 0.43, 0.39, and 0.45 respectively for alanine, valine-I, methionine, and tyrosine tRNA's. By the use of Eq. (1), r is calculated to be 0.79, 0.62, 0.68, and 0.59. The paired C/total C values determined from the infrared data for valine-I, methionine, and tyrosine tRNA's (0.62, 0.68, and 0.59, respectively) are in accord with what are expected on the "clover-leaf" models (0.60, 0.68, and 0.63, respectively) within the experimental error which would be about ± 0.05. The paired C/total C values determined from the infrared data of alanine tRNA of torula yeast (0.79) is not greatly different from what is expected on the "clover-leaf" model of alanine tRNA of baker's yeast (0.74).

In the spectral region of 1450—1480 cm^{-1}, poly(G+C) gives no absorptions, while polyAU-(copolymer with adenylic acid and uridylic acid in the alternating sequence) gives two bands here: a weaker band at 1480 cm^{-1} and a stronger band at 1460 cm^{-1}.[18] Therefore, from the spectral features of tRNA's in this spectral region an information on the content of the AU pair may be drawn. This is, however, yet to be done.

4) The 760—840 cm^{-1} Region. The absorptions in the 770 − 790 cm^{-1} region are assignable to out-of-plane vibrations of the base residues.[7] Poly (G+C) gives a strong band at 780 cm^{-1}, whose absorption intensity is more than three times of that of the 810 cm^{-1} band (skeletal stretching vibration around the 2'COH group of the ribose residue).[18] While, polyAU gives much weaker bands at 790 and 770 cm^{-1}.[18] The spectral features of the four tRNA's examined are somewhat different from one another (see Fig. 6). Thus, the spectrosopy in this region is also considered to be useful for an base analysis of tRNA's. This again is yet to be done.

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18) M. Tsuboi, Y. Kyogoku and S. Higuchi, to be published.