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Raman Spectra and Structure of Yeast Phenylalanine Transfer RNA in the Crystalline State and in Solution†

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ABSTRACT: Laser Raman spectra of yeast phenylalanine transfer RNA have been obtained in solution and in orthorhombic and hexagonal crystals. So far as one can tell from the spectra, which are identical in the two crystal forms, the molecular structure of the tRNA is not altered by differences in molecular packing in these two unit cells. In addition, the spectra of the two crystal forms show the same characteristic Raman frequencies and intensities as those of the tRNA in aqueous solution. Thus the structure of the tRNA molecule appears to be the same in the crystals and in aqueous solution. From the spectroscopic changes that result when Mg^{2+} ions are removed from the native tRNA, it is concluded that the removal of Mg^{2+} produces a partial disordering of the ribophosphate backbone of the molecule and a lowering of its melting temperature. The melting is shown to be a complex process in that the vibrations specific for adenine indicate a slightly lower melting temperature and those specific for guanine a slightly higher melting temperature than that of the ribophosphate backbone.

Detailed knowledge of the three-dimensional structure of macromolecules is obtained largely through X-ray diffraction studies of crystals. One of the central problems raised by studies of this type is the relationship between the three-dimensional structure revealed in the crystal study to the structure of the molecule in aqueous solution where the material is biologically active. During the past few years a great deal has been learned about the three-dimensional structure of yeast phenylalanine transfer RNA (tRNA^{Phe}) from X-ray diffraction studies. In the present report we make a comparison of the laser Raman spectra produced by this tRNA in the crystalline state and in a solution where it is biologically active. Since Raman spectra are sensitive to structure and molecular conformation, they represent a powerful method of comparing the molecule in these two states.

Yeast tRNA^{Phe} was used in X-ray diffraction studies because of the discovery 4 years ago that spermine complexes of this molecule produce crystals which yield high-resolution diffraction patterns (Kim et al., 1971). Over 2 years ago the electron density map at 4-Å resolution revealed that the molecules have L-shaped conformation with the acceptor and TψC stems of the familiar cloverleaf forming one arm of the L, while the dihydrouracil (D) stem and the an-

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ticodon stem form the other arm (Kim et al., 1973c). The distance between the 3′ terminal adenosine and the anticodon was found to be 76 Å. More recently, at 3-Å resolution in both the orthorhombic (Kim et al., 1974) and the monoclinic crystal forms (Robertus et al., 1974) of this molecule, further details were revealed concerning the tertiary interactions between nucleotides in the loop regions of the molecule. It is significant that the tertiary interactions are very similar in both the orthorhombic and the monoclinic unit cells, suggesting that the molecule has a stable, folded conformation independent of a particular crystallographic lattice.

The Raman spectra of nucleic acids and polynucleotides have been found specifically to yield information about the conformation of the ribophosphate backbone and the relative extent of base stacking and base pairing in both aqueous solution and the solid state (Hartman et al., 1973). The technique has been successfully applied to studies of various tRNAs in aqueous solution in different environments, for example, to give a quantitative estimate of the tRNA secondary structure in the native form and after thermal denaturation (Thomas et al., 1972, 1973; Small et al., 1972). Recent Raman studies by Chen and Thomas (1974) have shown that the backbone conformation of $tRNA^{Phe}$ as an amorphous powder is less sensitive to changes in the degree of hydration than is DNA.

In this paper we first present and discuss the temperature dependence of the Raman spectra of aqueous $tRNA^{Phe}$ in a native conformation and with Mg^{2+} ions removed. These spectra demonstrate well both the specificity of Raman spectroscopy and its sensitivity to structural change in tRNA. We next compare the spectra of crystalline and aqueous $tRNA^{Phe}$ to determine what, if any, structural changes are associated with the change of state. The spectroscopic results support the contention that the structure of $tRNA^{Phe}$ in aqueous solution is the same as in the crystals.

Experimental Methods

Yeast $tRNA^{Phe}$ was purchased from Boehringer-Mannheim Corporation. Orthorhombic crystals (about 300 μm × 70 μm) were grown (Kim et al., 1973a) at 4° in 40 m*M* sodium cacodylate buffer (pH 6.0), 40 m*M* $MgCl_2$, and 3 m*M* spermine·HCl in vapor-phase equilibration with 8% 2-propanol. 2-Propanol was removed from the crystals by vapor-phase equilibration with water, because it gives strong Raman lines that overlap certain tRNA lines. Control experiments were carried out to show that the X-ray diffraction patterns produced by the crystals in the absence of 2-propanol were virtually the same as in its presence. Hexagonal crystals (about 200 μm × 50 μm) were grown (Kim et al., 1973a) at 4° in 40 m*M* sodium cacodylate buffer (pH 6.0), 40 m*M* $MgCl_2$, and 2 m*M* $CoCl_2$ in vapor-phase equilibration with water.

For Raman measurements, Debye–Scherrer glass capillaries (0.6 mm i.d.) were first filled with the mother liquor, then the crystals were introduced into the capillaries and packed by centrifugation at 500*g* for 2 min to give a crystal layer about 2 mm thick. For the Raman spectra of solutions, the $tRNA^{Phe}$ was first dialyzed at 4° twice against 40 m*M* sodium cacodylate buffer (pH 6.0) containing 2 m*M* $MgCl_2$, and extensively against 2 m*M* $MgCl_2$ and then precipitated with 2 volumes of ethanol at −15°. The $tRNA^{Phe}$ was pelleted by centrifugation at 20,000*g* for 10 min, and the pellet was washed with acetone and dried over anhydrous $CaSO_4$ in a desiccator at 4°. A 3% solution of this $tRNA^{Phe}$ was prepared in doubly glass-distilled water or in the mother liquors of orthorhombic or hexagonal crystals; 3 μl of the solution was placed in a "Kimax" melting point capillary (1 mm i.d.) used as a Raman cell.

Raman spectra were recorded on a Spex Ramalog 4 double-grating spectrometer with 4880-Å excitation from a Coherent Radiation 52G Ar^+ laser. The scattered radiation was observed at 90° to the incident beam. All spectra were obtained at a spectral slit width of 8 cm^{-1}, period, 5 sec, scan speed, 1 cm^{-1}/sec. Frequencies for sharp lines are accurate to ±1 cm^{-1} and broad lines ±2 cm^{-1}.

In order to examine whether the crystals had undergone any dissolution due to heating by the laser beam, as soon as a spectrum of the crystals had been recorded, the spectrum of the mother liquor was obtained with the laser beam positioned 1–2 mm away from the crystal layer. The spectrum so obtained was identical with that of the mother liquor in the absence of crystals when the exposure of the crystals to 4880-Å radiation was no longer than the time of recording one spectrum of the orthorhombic crystals (~40 min at 25°). Exposures of the hexagonal crystals could be several times longer without significant effect. This indicates the stability of the $tRNA^{Phe}$ crystals under the conditions of observation described above.

Laser irradiation was also shown to have no effect on the charging activity of the $tRNA^{Phe}$ solution for phenylalanine, which was the same before and after Raman spectra were recorded, as assayed by standard methods with a crude yeast enzyme preparation (Giegé et al., 1972).

Low magnesium $tRNA^{Phe}$ was prepared by heating $tRNA^{Phe}$ (15 mg/ml) at 90° for 10 min in 20 m*M* Na-EDTA (pH 7.0). After the mixture was cooled to room temperature the tRNA was dialyzed overnight against 20 m*M* Tris-HCl (pH 8.0), then dialyzed four times, twice each for 6 hr against 40 m*M* and 5 m*M* sodium acetate (pH 5.0). All dialyses were done at 4° in a plastic beaker and in the presence of "Chelex" resin. All vessels and Raman cells used had been extensively washed with EDTA and doubly glass-distilled water.

Raman cells were placed in a thermostat similar to that of Thomas and Barylski (1970), by means of which temperatures can be controlled in the range of 10–90° to a constancy of 0.5° and an accuracy of better than ±1°. Spectra were obtained in one continuous recording without disturbing the Raman cell; 30 min were allowed for the cell to reach equilibrium after the thermostat temperature had become constant at a new temperature.

Results and Discussion

Effects of Removal of Mg^{2+} Ions at 25°. Original Raman spectra of 3% native $tRNA^{Phe}$ at 25° and 3% low Mg^{2+}-$tRNA^{Phe}$ at 25 and 60° are reproduced respectively in Figures 1 and 2. In the latter the normalized spectra are redrawn together on a flat baseline to reveal more closely the results of thermal denaturation of the $tRNA^{Phe}$. The most significant intensity changes in the Raman spectra produced by removing Mg^{2+} from native $tRNA^{Phe}$ and by heating on low Mg^{2+}-$tRNA^{Phe}$ are listed in Table I. In the table the peak height of the line at 1100 cm^{-1}, above a base line drawn tangent at approximately 1065 and 1130 cm^{-1}, is taken as a standard equal to unity because its half-width of 23 cm^{-1} (full width at half-maximum) and integrated area have been found constant in all spectra (Thomas and Hartman, 1973; Thomas et al., 1972, 1973; Chen and Thomas, 1974; Brown et al., 1972). Use of the line at 1100

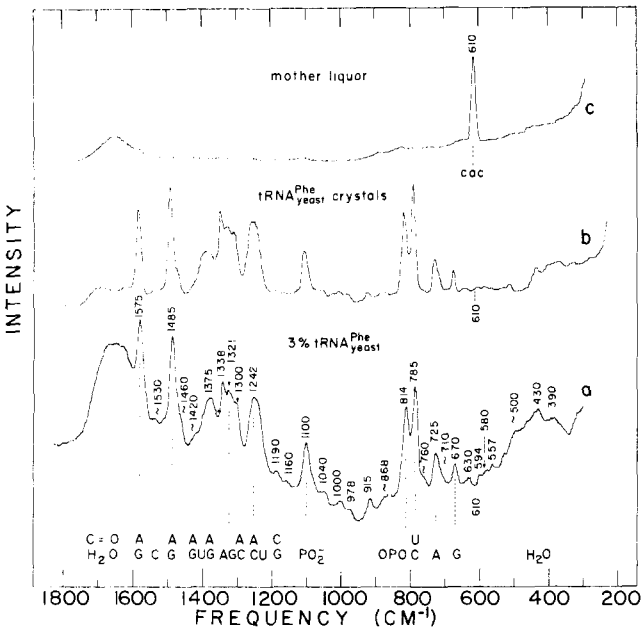

FIGURE 1: Original recording of Raman spectra of yeast tRNA^{Phe} at 25°: (a) 3% aqueous solution, beam power 300 mW; (b) orthorhombic crystals, beam power 40 mW; (c) mother liquor in which the crystals were immersed, beam power 120 mW. All spectra were recorded with identical instrumental conditions with the indicated laser powers measured at the sample. The origins of the Raman lines (Lord and Thomas, 1967; Shimanouchi et al., 1964) are indicated by A = adenine, U = uracil, G = guanine, C = cytosine, OPO and PO_2^- = phosphate, cac = cacodylate. When two bases simultaneously contribute to the same Raman line, the base which contributes more is placed below the other one. The dashed line at 610 cm^{-1} in spectra a and b indicates the position of the Raman scattering from cacodylate ion if it were present (see text).

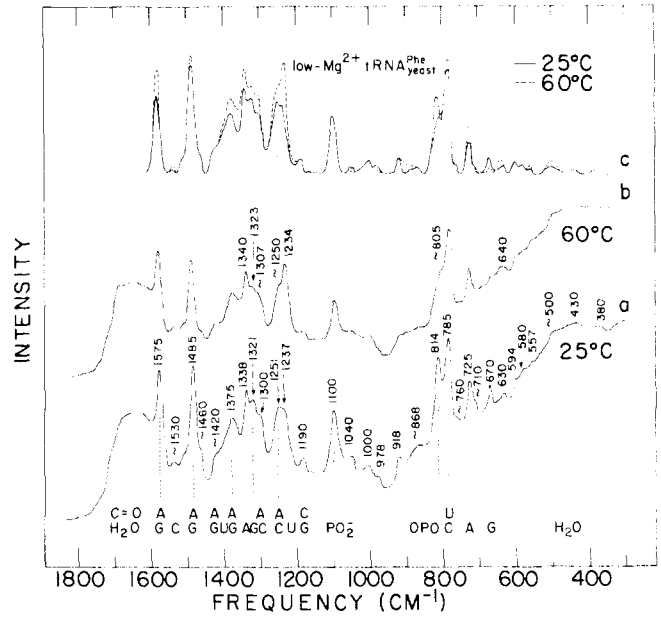

FIGURE 2: Original recording of Raman spectra of low Mg^{2+}-tRNA^{Phe}: (a) 25°; (b) 60°; (c) superposition of spectra a and b after correction for the water background and normalization to give the same intensity at 1100 cm^{-1}. Beam power at the sample 300 mW.

Table I: Frequencies and Intensities of Some Raman Lines of Aqueous tRNA^{Phe}, before and after Removing Mg^{2+} Ions.[a]

Frequency (cm^{-1})	Origin of Line	Expected ΔI	Relative Intensity: Native tRNA^{Phe} 25°	Relative Intensity: Low Mg^{2+} 25°	Relative Intensity: tRNA^{Phe} 60°
670	G	–	0.44	0.31	0.15
725	A	+	0.69	0.54	0.77
785	C and U	+	2.16	1.84	1.95
814	–OPO–		1.74	1.34	1.0 sh
1100	PO_2^-		1.00	1.00	1.00
1234	U	+	1.37	1.19	1.97
1251	C and A	+		1.27	1.5 sh
1300	C and A	+	1.1 sh	1.1 sh	1.4 sh
1321	G	+	1.34	1.29	1.55
1338	A	+	1.54	1.50	1.85
1375	G and A	+	1.07	1.03	1.31
1485	G and A	+	1.90	1.92	2.02
1575	G and A	+	1.40	1.36	1.79

[a] All solutions were 3% by weight. See text for discussion of table. All values are averages from at least five spectra of each of the two different batches of tRNA, and are within ±6% accuracy.

cm^{-1} as a standard assumes that its peak intensity does not change after removal of the Mg^{2+} ions (Table I, columns 4 and 5). The assumption seems reasonable in the light of the observation that a 100-fold change in Mg^{2+} concentration makes a relatively small difference in the line's peak intensity and virtually none in the integrated area (Thomas et al., 1973).

The relative peak heights of the other lines are given in columns 4, 5, and 6, with the estimated intensities of unresolved lines (marked sh, shoulder) rounded off. In the third column, ΔI, a plus sign signifies an expected increase ("hypochromism") and a minus sign an expected decrease ("hyperchromism") in the intensity of the line in question when the molecule is denatured (Small et al., 1972; Thomas et al., 1972, 1973; Chen and Thomas, 1974). A single plus sign for two bases together means that the sum of the two expected behaviors is positive.

Removal of Mg^{2+} from native tRNA^{Phe} produces a decrease of 23% in the intensity of the line at 814 cm^{-1}. This time arises from the symmetric O–P–O stretching vibration of the phosphodiester group (Shimanouchi et al., 1964), and is a sensitive measure of the ordered conformation of the RNA backbone (Brown et al., 1972; Thomas and Hartman, 1973). "Ordered conformation" here refers to the conformation of the C–O–P–O–C diester sequence in the ribose-phosphate backbone, in which the phosphodiester group must have the same geometry as in the double- or single-stranded ordered polyribonucleotides. On this basis the decrease in the intensity of the 814-cm^{-1} line in Figure 2a compared to Figure 1a is interpreted as a decrease in the number of C–O–P–O–C groups possessing the geometry that this group possesses in the native molecule. Thus we conclude that a partial disordering of the ribose–phosphate backbone is associated with the removal of Mg^{2+} from the native tRNA.

The magnitude of this disordering is substantial and leads to the conclusion that structural change occurs whenever treatment of tRNA^{Phe}, however benign in other respects, diminishes its Mg^{2+} content. Thus one must be careful when assessing the order of a sample of tRNA by the intensity of the Raman line at 814 cm^{-1} to make sure that Mg^{2+} ions have not been depleted by purification procedures. A number of determinations of order in tRNAs based on the ratio I_{814}/I_{1100} have appeared in the literature (Small et al., 1972; Thomas et al., 1972, 1973; Chen and Thomas, 1974; Thomas and Hartman, 1973). In some of these (Small et al., 1972; Thomas and Hartman, 1973) or-

dered structures are taken to be those with the above ratio at about 1.7.

However, when a departure from this value of the ratio is attributed to disorder, it is not always clear whether the disorder is produced by depletion of Mg^{2+} or by some other form of denaturation. Thus our results show that the spectroscopic measurement of disorder should be made in such a way as to distinguish between loss of Mg^{2+} and other causes of disorder. Since physiological conditions supply sufficient Mg^{2+} to give the native amount of order to the ribose–phosphate backbone as measured by the Raman spectra, purification of tRNAs should proceed in such a way as to maintain their Mg^{2+} content if retention of this conformation is desired.

In addition to changes in the phosphodiester line, removal of Mg^{2+} leads to large intensity changes in the frequencies of certain planar vibrations of the bases, specifically those at 670 (due to the G bases), 725 (A), and 785 cm^{-1} (C and U). The intensities of these lines in the spectrum of native $tRNA^{Phe}$ at a given concentration depend linearly on the number of bases in stacked and in unstacked conformation. When the molecule goes from the native form to one that is completely disordered with all the bases in an unstacked conformation, the intensities change in proportion to the number of bases that change from the stacked to unstacked form. For example, the line at 670 cm^{-1} has been shown to exhibit inverse hypochromism, that is, its intensity decreases when the G bases from which it arises change from an ordered (stacked) configuration to an unstacked one (Small and Peticolas, 1971b). Thus the decrease of 30% shown in Table I is in the expected direction and suggests that a substantial number of G bases known to be stacked in the crystal have become unstacked by removal of Mg^{2+}. These are probably the three G's at the corner of the L-shaped structure which are in a stacked conformation (G18, G19, and G57).

On the contrary, the intensities in the lines at 725 and 785 cm^{-1} are known to be hypochromic and should therefore increase if the A, C, and U bases change from a stacked to an unstacked configuration. Surprisingly, the intensities decrease by about 20% on removal of Mg^{2+} ions (Table I), which implies an increase in the stacking of A and either C or U or both.[1] If because of pairing the stacking of the C bases should correlate to some extent with that of G, and stacking of U with A, the net effect of the expected decrease in stacking of C and increase in stacking of U on removal of Mg^{2+} should be a partial cancellation of the intensity change in the 785-cm^{-1} line. This is in fact observed: the percentage change at 785 cm^{-1}, −15%, is considerably smaller than those at 670 and 725 cm^{-1}, −30 and −22%, respectively. Moreover a small part of the apparent decrease at 785 cm^{-1} may arise from the substantial decrease in the overlapping OPO line at 814 cm^{-1}.

Above 1200 cm^{-1} there are eight prominent lines due to in-plane ring vibrations in the various bases. These lines are all hypochromic (Small and Peticolas, 1971a,b; Lafleur et al., 1972) but not strongly so except for that due to U at 1234 cm^{-1}. Removal of Mg^{2+} makes minor changes in their intensities that are within experimental uncertainty. A possible exception is the doublet at 1234–1251 cm^{-1}. With a spectral slit width of 8 cm^{-1}, this doublet is only partially resolved in the low Mg^{2+} form and not at all in native $tRNA^{Phe}$. It is therefore difficult to judge whether small changes in intensity have taken place. The effect of substantial structural change on this doublet will be shown later.

When Mg^{2+} ions are restored to the low Mg^{2+}-$tRNA^{Phe}$, the spectra are the same, apart from small quantitative differences that are within experimental error, as those of the original native samples, that is, the intensities based on the intensity 1.00 for the line at 1100 cm^{-1} agree with those of Table I, column 4. Interestingly, we have obtained a similar result by renaturing low Mg^{2+}-$tRNA^{Phe}$ with Na^+ or Mn^{2+} ions (Schreier and Schimmel, 1974; Cohn et al., 1969), although the accuracy of the intensities in the latter case is somewhat lower because of a slight fluorescent background due to Mn^{2+}.

The conclusion to be drawn from these results is that some structural change occurs when Mg^{2+} is removed from the $tRNA^{Phe}$ molecule at 25°, partly a small amount of disordering of the ribose–phosphate backbone and partly an unstacking of about one-third of those G bases that become unstacked at 60°. This appears to be accompanied by some increase in the adenine stacking. A small decrease in the C and U line intensity at 785 cm^{-1} is consistent with these changes in the G and A stacking.

Thermal Denaturation of Native and Low Mg^{2+}-$tRNA^{Phe}$. When aqueous native yeast $tRNA^{Phe}$ is heated to temperatures above 70° at concentrations of 3% or higher, the solution becomes cloudy, presumably as a result of molecular aggregation. This behavior is reversible. It is different from that of *Escherichia coli* $tRNA^{Glu}$ and $tRNA^{Val}$, both of which yield clear, spectroscopically satisfactory solutions (Thomas et al., 1972, 1973) at 90°. Because quantitative measurement of Raman intensities from turbid solutions is difficult, we have not used this technique with native $tRNA^{Phe}$ above 70°. Below 70°, however, the intensity variation with temperature is similar to that found for low Mg^{2+}-$tRNA^{Phe}$ (see below) except for a shift of the $I(t)$ curve to higher temperatures by about 18°. These results suggest that the melting temperature, T_m, of native $tRNA^{Phe}$, if measured by the intensity of the Raman line at 814 cm^{-1}, should be about 72°, in agreement with that obtained by ultraviolet measurements (Riesner and Römer, 1973).

When Mg^{2+} is removed from $tRNA^{Phe}$, turbidity no longer appears at elevated temperatures and acceptable spectra can be obtained (Figure 2b). A clear-cut effect of low Mg^{2+} is a reduction in the T_m. This can best be determined, when Raman spectroscopy is employed, by following the intensity of the phosphodiester line at 814 cm^{-1}. The temperature dependence of this intensity is shown in Figure 3. Since there is considerable overlap between the two lines at 785 and 814 cm^{-1}, which results in an apparent frequency shift of the latter to 805 cm^{-1} when the solution is heated from 25 to 70°, the *integrated intensities* of the lines have been normalized to that of the line at 1100 cm^{-1} on the assumption that the line shapes are gaussian. For the temperature dependence of two other lines, 670 and 725 cm^{-1}, the *peak heights* are used because there is very little

[1] The measurement of stacking of adenine by means of the line at 725 cm^{-1} is complicated by the existence of an underlying line (Thomas et al., 1973) at almost the same frequency (721 cm^{-1}) due to dihydrouracil. On a molar basis this line is about twice as intense as the adenine line, and since there are nine times as many adenines (RajBhandary and Chang, 1968), the dihydrouracils should contribute about 22% of the total intensity. Since the effect of stacking on the intensity of the dihydrouracil line is not known at present, we assume the effect of dihydrouracil on the intensity change to be the same as that of the adenine, which may lead to an error as large as 20% in the quantitative estimate of the change in the stacking of adenine.

interference from the neighboring lines and the half-widths change little if at all when the temperature of the solution is raised from 25 to 70°.

Much research has been done on the denaturation of various tRNAs by temperature. Usually thermal transition curves are obtained with the help of the hypochromic effect on ultraviolet absorption (Riesner and Römer, 1973), though this has the disadvantage that the effects of the different bases are superimposed. In nuclear magnetic resonance (NMR) studies hydrogen-bonding protons connecting the different kinds of base pairs give interpretable chemical shifts in favorable cases, but the temperature dependence of the NMR spectra is difficult to interpret in terms of the behavior of the individual bases (Kearns and Shulman, 1974).

The temperature dependence of the Raman spectra provides additional information to that from the uv and NMR. In Figure 3 are presented the variations in intensity with temperature of those Raman lines from the various bases that show appreciable hypo- or hyperchromism. The different temperature dependences observed imply a sequential unfolding of the tRNA^{Phe}. The unstacking of the adenines (curve A in Figure 3) is found to occur on the average at a somewhat lower temperature (45.5°) than that of the other bases, of which the guanines are the last to unstack (56.5°). To the extent that the stacked A and G are paired respectively with U and C, the temperature variation of U and C, as shown by the Raman line at 785 cm^{-1}, has the expected behavior, since the temperature dependence of the line ("unstacking" temperature of 50.0°) is intermediate between that of A and that of G.

Additional information is obtained independently from the observation of the "melting" of the ribose-phosphate backbone by means of the line at 814 cm^{-1}. The temperature dependence of this line gives a melting temperature T_m of 53.5° (curve (OPO) in Figure 3). The curve shows a gradual transformation from "ordered" to "disordered" conformation; thus the transition is not a cooperative one. This is in accordance with the sequential melting inferred from the separate unstacking of the various bases. It is also worth noting that a change in temperature from 25 to 40° produces an observable decrease (about 10%) in the relative intensity of the 814-cm^{-1} line. That is, a measurable amount of backbone "disorder" results from this temperature change, though the percentage of disordered phosphodiester links is small (presumably about 10% of the total phosphate groups). This quantitative point will be useful later in comparing the spectra of crystalline and aqueous tRNA^{Phe}.

The intensities of the frequencies above 1200 cm^{-1} in Table I vary with temperature in the manner expected from the behavior of the bases to which they are due. The region 1150–1450 cm^{-1}, whose intensity pattern may be regarded as the "fingerprint" of the particular tRNA, is highly characteristic of the base composition (Thomas et al., 1973). Unfortunately it consists of lines that overlap strongly and are therefore rather less suitable than those of Figure 3 for quantitative measurement of stacking, though the qualitative changes with temperature are clear enough (Figure 2).

Finally it should be mentioned that the spectra in Figure 2 and the curves in Figure 3 are reversible when the temperature is raised and lowered through several cycles between 25 and 70°.

These experiments illustrate the power of laser Raman spectroscopy to monitor different regions of a macromole-

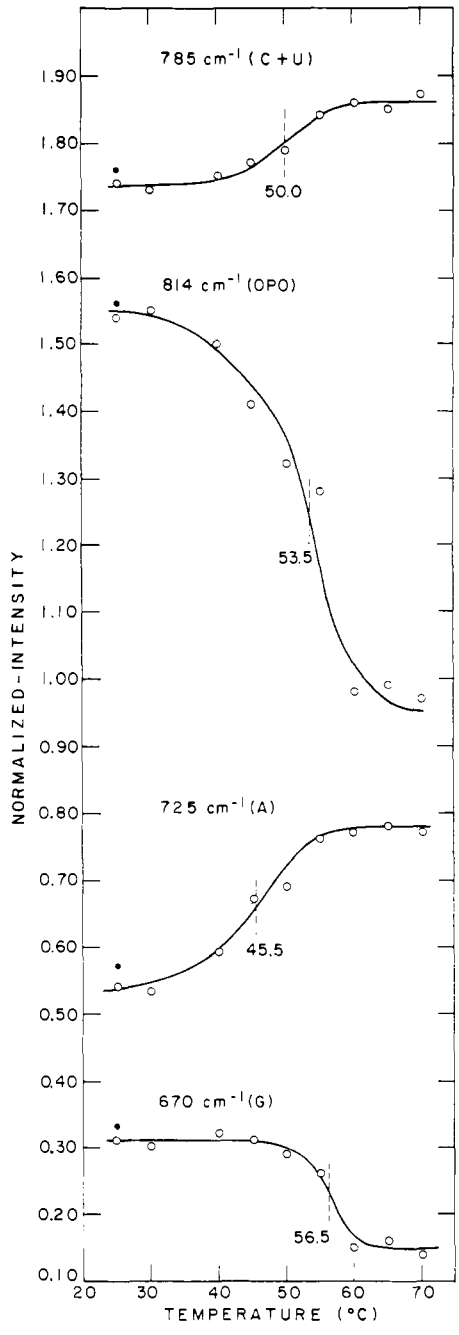

FIGURE 3: Thermal transition curves of low Mg^{2+}-tRNA^{Phe} as monitored by the lines at 670, 725, 785, and 814 cm^{-1}. The melting temperatures of transitions are also indicated. Solid symbols represent measurements after cooling the solution from 70 to 25° over a period of approximately 3 hr. Letters in parentheses denote the origin of the Raman line in question.

cule during a transition. Although we do not know the detailed conformation of the molecule in the low Mg^{2+} form, it is likely that the ends of the L-shaped molecule will remain free as they are in the crystalline state. It is interesting that the CCA end and the anticondon loop contain a total of 5 adenine residues, with 13 other adenines in the center of the molecule. It is quite possible that the initial step in the thermal melting is the unstacking of adenine residues at the two ends of the molecule where they are not held together by secondary or tertiary hydrogen-bonding interactions. Since these ends constitute "adenine-rich" regions, this would be reflected in a lower T_m for the adenines. Earlier studies of spin-labeled tRNAs were carried out in which

Table II: Intensities of Some Raman Lines of Yeast $tRNA^{Phe}$ [a]

Frequency (cm^{-1})	Origin of Line	Aqueous Solution	Orthorhombic Crystals	Hexagonal Crystals
670	G	0.44	0.45	0.47
725	A	0.69	0.68	0.72
785	C and U	2.16	2.10	2.09
814	–OPO–	1.74	1.70	1.72
1100	PO_2^-	1.00	1.00	1.00

[a] Peak heights in each spectrum are normalized to the height of the 1100-cm^{-1} line. All values are averages from at least five spectra of three different batches of tRNA, and are within ±6% accuracy. Intensities of other lines in the spectra show similar constancy within the stated experimental error.

the spin-label was attached to the α-amino group of *E. coli* valyl-$tRNA^{Val}$ or phenylalanyl-$tRNA^{Phe}$ (Hoffman et al., 1969; Schofield et al., 1970). Melting studies of these systems also showed that the spin was labilized at a lower temperature than the temperature at which the maximal uv spectral changes occurred.

Similarly, the fact that the guanine residues exhibit a T_m slightly higher than the remainder of the molecule is as expected. Most of the guanine residues are paired to cytosines (14 out of 22) and the higher T_m of polynucleotides with G–C pairs is well documented.

Comparison of Crystal and Solution Spectra. The foregoing data and conclusions show that many lines in the tRNA spectrum are sensitive to the tRNA conformation under various environments It is therefore of particular interest to determine the effect of the aqueous solvent on the conformation of yeast $tRNA^{Phe}$, whose structure in the crystal has been the subject of intensive studies by X-ray diffraction (Kim et al., 1974; Robertus et al., 1974). The Raman spectra of randomly oriented orthorhombic crystals of $tRNA^{Phe}$ and the 3% aqueous solution are presented in Figure 1. The hexagonal crystals give essentially the same spectrum as the orthorhombic, and the relative intensities of some of the lines are tabulated in Table II.

Furthermore, when the crystals are dissolved in their respective mother liquors to a concentration of 3% by weight, the Raman spectra are identical with that of native $tRNA^{Phe}$ in 3% aqueous solution (Figure 1a), except for the appearance of a line at 610 cm^{-1} due to cacodylate. This means that the mother liquor in which the crystals were immersed does not cause any detectable structural alteration in the $tRNA^{Phe}$.

The most striking feature of Figure 1 and Table II, in view of its structural implication, is the relatively small difference, after correction for water background between the spectrum of the aqueous solution and that of the crystals. The frequency and relative intensity of the 814-cm^{-1} line, which is sensitive to conformational change in phosphodiester backbone of the tRNA, are identical within experimental reproducibility. Other lines in the spectra are likewise constant. This result strongly supports the structural similarity of yeast $tRNA^{Phe}$ in the crystalline state and in the solution, so far as one can conclude from Raman spectroscopy.

Crystallization improves considerably the background of the spectra of $tRNA^{Phe}$ (Figure 1a and 1b). The lines at 1300 and 1338 cm^{-1} are somewhat sharper, and the valley between the two lines at 785 and 814 cm^{-1} is deeper in the spectrum of the crystals than in that of the solution. In the latter, there is weak scattering by H_2O at 800 cm^{-1} that can partially fill in this valley. More importantly, the slightly broader lines of the aqueous spectrum probably indicate perturbation of the vibrating molecular groups by water molecules, whose intermolecular effects on the frequencies vary more widely than those produced by the fewer and more constrained water molecules in the crystals. Attention should also be called to the difference of a factor of 8 in the laser power used for the spectra in Figure 1a and 1b. Although the crystals contain about 70% water (Kim et al., 1973b), the volume concentration of $tRNA^{Phe}$ is still a factor of 10 higher than in the 3% solution. The absence of a clean-cut water background in Figure 1b results from the smaller power needed to get a $tRNA^{Phe}$ spectrum quantitatively comparable to that of the solution in Figure 1a.

Since the crystals are grown from a mother liquor containing 40 m*M* sodium cacodylate buffer, it is important to know whether the cacodylate ion enters the crystals as an integral part of the structure. Because cacodylate ion is a strong scatterer (Figure 1c), it is readily possible to determine its concentration by means of the Raman effect. Spectrum c in Figure 1 is that of the mother liquor obtained with the laser beam shifted 1–2 mm away from the crystals, as described above under Experimental Methods. The strong line at 610 cm^{-1}, due to the cacodylate ion, cannot be detected in the spectrum of the crystals (Figure 1b). To escape detection under the conditions of observation, the concentration of cacodylate ion in the crystal should be 5 ± 2.5 m*M*, whereas the crystal density corresponds to a concentration of 22 m*M* of $tRNA^{Phe}$ (Kim et al., 1971). Thus there is an upper limit of about ⅓ mol of cacodylate ion that can be present per mol of $tRNA^{Phe}$ in the packed crystals.

Recently it has been reported that the three-dimensional structure of yeast $tRNA^{Phe}$ in the monoclinic crystal is essentially the same as that in the orthorhombic crystal (Quigley et al., 1974; Klug et al., 1974). Thus with the results furnished in the present study, it can further be concluded that the structure of yeast $tRNA^{Phe}$ in the monoclinic unit cell is very similar to that of the hexagonal crystal and that in aqueous solution.

When the Y base is removed from the anticondon loop of $tRNA^{Phe}$ by the method of Li et al. (1973), we found that the Raman spectrum is essentially unchanged from that of the native molecule within our experimental reproducibility. Thus the structural changes resulting from the removal of the Y base appear to be rather small so far as Raman spectroscopy can determine, although it has been concluded from NMR studies (Kearns et al., 1973) that this removal increases all the interbase separations in the anticondon stem by about 0.2 Å. Such small changes do not affect the overall Raman intensities, just as they do not affect the overall hypochromicity (Kearns et al., 1973). Similarly, it has recently been reported by Prinz et al. (1974) that yeast $tRNA^{Phe}$ shows changes in its circular dichroism when treated with the solvent mixtures used in crystallization for X-ray studies. The dichroic changes observed are rather small and perhaps imply structural changes too small to be detected by the Raman effect. However, as discussed above, native $tRNA^{Phe}$ in aqueous solution gives the same Raman spectrum as in the crystals (Figure 1), whereas the removal of Mg^{2+} gives a substantially altered spectrum (Table I) and melting curves (Figure 3).

In conclusion, the spectroscopic results described in this paper provide added support for the postulate that the three-dimensional structure of yeast $tRNA^{Phe}$ is the same

in solution as it is in the orthorhombic crystals which were used for the X-ray diffraction studies.

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