TRANSFER RNA: CHANGE OF CONFORMATION UPON AMINOACYLATION DETERMINED BY RAMAN SPECTROSCOPY*

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SUMMARY: Laser-excited Raman spectra of yeast tRNAs in charged and uncharged states are compared to reveal differences in base stacking. Aminoacylation of unfractionated tRNA reduces considerably the amount of stacking of both adenine and pyrimidine residues. In Phe-tRNA^{Phe}, however, only the adenine residues appear to be significantly less stacked after aminoacylation. The overall degree of order in the backbone of yeast tRNAs is little affected by these changes in base-stacked secondary structure.

Aminoacylation of transfer ribonucleic acid (tRNA) alters its interactions with aminoacyl-tRNA synthetase, Tu-factor, f2-factor, histidine operon repressor and ribosomal binding sites (1). These differences in function of charged and uncharged tRNA suggest a change in the molecular conformation upon aminoacylation. Other indirect evidence has been given to support this view (2).

Recent experiments to determine if conformational differences exist between charged and uncharged tRNAs have yielded conflicting results. For example, some CD and UV studies of $tRNA^{Val}_{\underline{E.coli}}$ and $tRNA^{fMet}_{\underline{E.coli}}$ (3) have been interpreted in favor of no significant changes in secondary structure (base-pairing or base-stacking) upon charging, while other CD and UV studies of $tRNA^{fMet}_{\underline{E.coli}}$ (4) give evidence of distinct conformational changes accompanying

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aminoacylation. Relaxation kinetic studies of the binding of ethidium bromide to unfractionated tRNA (5) reveal a conformation change upon aminoacylation which can be completely reversed by deacylation. Similar observations have been made by proton magnetic resonance (p.m.r.) measurement of the longitudinal relaxation of solvent H_2O protons by Mn^{2+} bound to tRNA (6). On the other hand, major changes in conformation from aminoacylation have not been detected in partial nuclease-digestion studies of $tRNA^{Phe}_{E.coli}$, $tRNA^{Phe}_{yeast}$, and $tRNA^{Ser}_{yeast}$ (7); in small-angle X-ray scattering studies of $tRNA^{Val}_{E.coli}$ (8); or in the rates of hydrogen-tritium exchange of $tRNA^{fMet}_{E.coli}$ (9). Despite such conflicting evidence, the majority of investigators conclude that small changes in tertiary structure may accompany aminoacylation of tRNAs (8).

Previous studies of rRNA and tRNA by Raman spectroscopy (10-13) have provided detailed information on their primary, secondary and tertiary structures in aqueous solution. We have, therefore, examined the Raman spectra of yeast tRNA in charged and uncharged states to determine whether or not detectable conformational changes accompany aminoacylation and whether the molecular subgroups affected may be identified. The yeast tRNAs on which we report here are $tRNA^{Phe}$ and unfractionated tRNA (minus $tRNA^{Phe}$).**

The procedure for separating and purifying $tRNA^{Phe}$ and $tRNA^{u}$ as the potassium salts from crude unfractionated yeast tRNA (Sigma, Type III, Lot No. 110C-8100) was that of Gillam et al. (14). Aminoacylated $tRNA^{u}$ was prepared and the extent of reaction monitored as described previously (5). The reaction mixture contained in a total volume of 5 ml: 50 mg $tRNA^{u}$, 0.2 mM each 20 mixed amino acids, 10 mM ATP, 50 mM $MgCl_2$, 100 mM tris-HCl (pH 7.5), 65 mg partially purified yeast aminoacyl-tRNA synthetases and 10 mM CTP. The reaction was initiated by adding enzyme. The mixture was incubated for 20 min at 25°C, chilled in ice for 1 min, extracted with phenol by vigorous shaking for 10 min and centrifuged to separate phases. The aa-$tRNA^{u}$ was pre-

**Abbreviations used: $tRNA^{u}$-unfractionated yeast tRNA (minus $tRNA^{Phe}$); aa-$tRNA^{u}$-the aminoacylated form of this material.

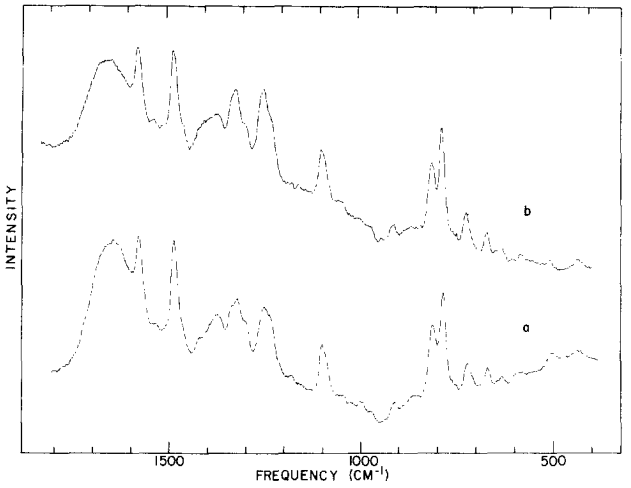

Figure 1. Original recordings of Raman spectra (a) $tRNA^{u}$ and (b) $aa\text{-}tRNA^{u}$. Conditions: 40μg RNA/μl H_2O, 32°C, no added salts ($[K^+] \approx$ 0.12M); 4880Å excitation (200 mW), slit width 10 cm^{-1}, scan rate 25 cm^{-1}/ min, period 20 sec. The spectrum of an equimolar mixture of 20 mixed amino acids and $tRNA^{u}$ is identical to (a).

cipitated with ethanol, redissolved in H_2O, dialyzed in the cold against 1 mM EDTA (200 volumes with two changes at 2-hour intervals) and then double-distilled H_2O (4 changes at 12-hour intervals), lyophilized from solution and stored at -20°C until solutions were prepared for Raman spectroscopy. A similar procedure was employed for preparing the potassium salt of $Phe\text{-}tRNA^{Phe}$ essentially free of Mg^{2+}. Under these conditions spontaneous hydrolysis of the aminoacyl-tRNA ester bonds should be minimal (15) and a control experiment using [^{14}C]phenylalanine indicated a half-life of more than one week. Purified samples of $tRNA^{u}$ and $tRNA^{Phe}$ were dialyzed and lyophilized in the same way as the corresponding charged species.

Sample-handling and instrumentation for Raman spectroscopy of tRNAs have been described (10-12). Other conditions are given in the legend of Fig. 1.

In Figs. 1a and 1b we show the Raman spectra of $tRNA^{u}$ and $aa\text{-}tRNA^{u}$, respectively. The intensity changes which result from aminoacylation are revealed more clearly in Fig. 2 where the spectra have been normalized and redrawn over a flat baseline. Qualitatively similar results are obtained for $tRNA^{Phe}$ and $Phe\text{-}tRNA^{Phe}$.

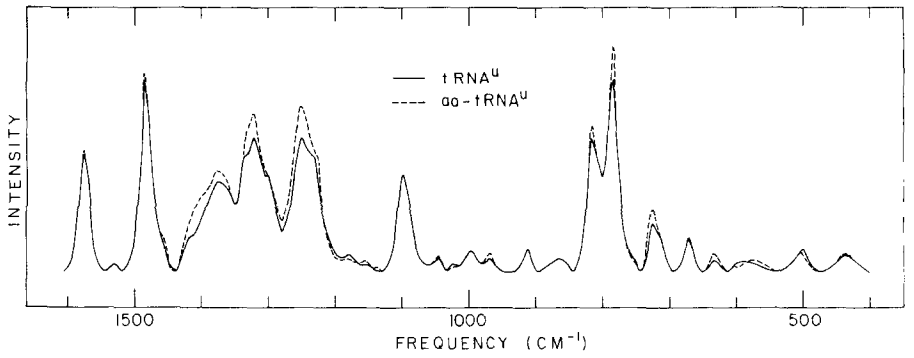

Figure 2. Raman spectra (400-1600 cm ^{1}) of tRNA^{u} and aa-tRNA^{u} normalized to give the same intensity at 1100 cm^{-1}, redrawn over a flat base-line and corrected for the Raman scattering of liquid H_2O.

The Raman lines which are intense, well-resolved and unambigously assignable to tRNA subgroups (16) are listed in Table I, where relative intensities are compared for charged and uncharged states of tRNA^{u} and tRNA^{Phe}. For tRNA^{u}, charging produces significant increases in the intensities at 725 and 785 cm^{-1}, while the increase at 814 cm^{-1} is close to the experimental uncertainty in view of the overlap with the peak at 785 cm^{-1}. For tRNA^{Phe} the results are analogous to those of tRNA^{u} with the exception that no significant increase in intensity occurs at 785 cm^{-1}. Spectra of equimolar mixtures of amino acid + tRNA were indistinguishable from spectra of the uncharged tRNAs. Thus the observed changes must result from alteration of the conformation of the tRNAs by aminoacylation and cannot be due, for example, to a mixture of the original tRNA and amino acids resulting from spontaneous hydrolysis of the charged tRNAs during purification or spectroscopic study.

We interpret these results as follows. In polyribonucleotides the Raman lines at 725 and 785 cm^{-1} are hypochromic with respect to the stacking of A and C+U residues, respectively. For example, in the ordered single-stranded structure of poly(rA), in which each A is stacked between adjacent A residues, the 725 cm^{-1} line is 50% less intense than in the random-chain configuration of poly(rA) (17). The hypochromism at 725 cm^{-1} is the same for poly(rA)·

poly(rU) (18) in which the A residues are paired to U as well as stacked between adjacent A residues, and for poly(rA-rU)·poly(rA-rU) (19) in which the paired A residues are stacked between adjacent U residues. For partially ordered molecules, such as 16S and 23S rRNA, the hypochromism at 725 cm^{-1} is, as expected, smaller in magnitude than in the completely helical polyribonucleotides (20). Similarly, hypochromism is observed for the 785 cm^{-1} line when C and/or U residues occur in stacked configurations in polynucleotides, (17) in rRNAs (11,20) and in tRNAs (12,16). It is thus well established that these hypochromic effects occur in hetero- as well as homopolyribonucleotides of varying complexity and are a realistic measure of base-stacking. Therefore, the intensity changes at 725 and 785 cm^{-1} in $tRNA^{u}$ mean that charging produces a decrease in the extent of stacking of adenine and of pyrimidine residues. For $tRNA^{Phe}$, charging produces significant unstacking of A residues only. The Raman line at 814 cm^{-1}, which derives its intensity exclusively from ordered phosphodiester linkages (21), indicates a small but probably real increase in the amount of order of the tRNA backbones upon charging. The spectral changes observed in the region 1150-1450 cm^{-1} for $tRNA^{u}$ (Fig. 1) are consistent with these conclusions (16).

In summary, the present results show that the preparations of $tRNA^{Phe}$ and $tRNA^{u}$ undergo significant changes in secondary structure upon aminoacylation. At the conditions employed, the charged tRNAs exhibit less base-stacked secondary structure than the uncharged tRNAs, but the overall degree of order in the tRNA backbones is not decreased by aminoacylation.

The differences in structure between $tRNA^{u}$ and aa-$tRNA^{u}$ are greater than those between $tRNA^{Phe}$ and Phe-$tRNA^{Phe}$ (Table I). We therefore conclude that the structural effects of aminoacylation probably differ among various tRNAs. This fact could explain the apparently conflicting results obtained by different investigators (2-4,7-9) who have studied the structural effects of charging different tRNAs. Alternatively the differences between tRNAs may

Table I

INTENSITIES OF RAMAN LINES IN CHARGED AND UNCHARGED tRNAs[a]

Position in cm^{-1} (assignment)	$tRNA^{u}$	aa-$tRNA^{u}$	% change[b]	$tRNA^{Phe}$	Phe-$tRNA^{Phe}$	% change[b]
670(G)	0.45	0.43	---	0.26	0.28	---
725(A)	0.51	0.66	+29	0.63	0.76	+21
785(C+U)	2.01	2.31	+15	2.20	2.08	---
814(P)	1.36	1.52	+12	1.20	1.35	+12
1100(P)	1.00	1.00	---	1.00	1.00	---
1485(G+A)	1.92	2.00	---	1.73	1.74	---
1575(G+A)	1.22	1.24	---	1.10	1.15	---

[a] Intensities in each spectrum are normalized to the 1100 cm^{-1} peak height (10). Values cited are averages from four spectra of each of two independently prepared samples (average deviations are within ±5%).

[b] Changes of 10% or less are within experimental uncertainty and are denoted by a dash. It is shown in ref. 21 that the ratio of intensities at 814 and 1100 cm^{-1} gives quantitatively the total secondary structure of aqueous RNA, provided excess counterions (Na^{+}) and Mg^{2+} are absent. Since such conditions may not be fulfilled here, we have restricted the discussion in the text to a semi-quantitative level.

reflect varying rates of spontaneous hydrolysis of aa-tRNA depending upon the nature of the attached amino acid (15).

Further Raman studies are in progress to determine the effects of aminoacylation on other purified tRNAs and to improve the sensitivity of the method. For example, it will be of interest to determine intensities of the Raman line at 814 cm^{-1} (Table I) more precisely to ascertain whether changes in this line are sufficiently definite to imply clear-cut changes in the backbone conformations of these tRNAs.

After the present paper was prepared for publication, we received a preprint of the paper of Wong, Read and Kearns (22) describing a study by high-resolution p.m.r. spectroscopy of the effect of charging on the conformation of yeast $tRNA^{Phe}$. These authors found nearly identical spectra for

charged and uncharged molecules and concluded that charging produces little if any structural change. Their observations, however, were confined to the hydrogen-bonded NH protons of the paired bases of the tRNA. Our results are in accord with theirs in that we confirm the stability of the GC structure to charging and also find that the overall order in the phosphate-sugar backbone is little affected. Since the p.m.r. technique appears to be less sensitive to conformational effects on the unpaired bases, the p.m.r. spectra are not in conflict with our observation of intensity changes associated with some unstacking of the adenines, provided these are not paired in the uncharged tRNA. Our observation that the effects of charging are much greater for $tRNA^{u}$ than for $tRNA^{Phe}$ clearly makes it important to identify those charged components of $tRNA^{u}$ that do show substantial structural change by Raman spectroscopy and to examine these by p.m.r. spectroscopy and other techniques.

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