NMR STUDY OF THE MODIFIED BASE RESONANCES OF $tRNA^{tyr}_{coli}$

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SUMMARY

220MHz NMR spectra at 28° show several resolved resonances in the high field region for D_2O solutions of tyrosine specific tRNA from E. coli. These resonances are tentatively identified as arising from protons of the modified nucleoside, 2-methylthio-N^6-(Δ^2-isopentenyl)-adenosine and from the modified guanosine of unknown structure in the "wobble position" of the anti codon loop. Assignment of resonances was aided by comparison with spectra of $tRNA^{tyr}_{su^+_{III}}$, Form II, whose sequence is closely homologous to $tRNA^{tyr}_{coli}$, except for changes in some modified bases. Line widths of resolved resonances indicate that, at 28°, the methyl groups of modified nucleosides are not completely restricted in their motion relative to the overall motion of the macromolecule.

Proton magnetic resonance spectra of transfer RNA's in D_2O solution generally show poorly resolved resonance bands (1-3). Several techniques have been applied towards narrowing and resolving individual resonances including elevated temperatures (1-3) and denaturation in dimethyl sulfoxide (4,5). Exchangeable, hydrogen-bonded protons may be studied in H_2O solution (6,7) and this has provided another useful approach. Finally, attention has been focussed on the spectral region where resonances appear due to the modified nucleosides in tRNA (3,5).

Even in this high field region, broad, overlapping resonances have been observed (3,5). Since the overlap is due, in large part, to the abundance of modified bases in the particular tRNA's studied, interpretation would be made easier by choosing a tRNA with very few modifications. Transfer RNA^{tyr} from E. coli is a very attractive molecule for study from this viewpoint since only 4 residues have potential resonances in the high field region: ribothymidine at position 63, 2'-O-methyl G at position 17, a modified G of unknown structure (designated G*(8) or Q(13)) at posi-

tion 35, and N^6-(Δ^2-isopentenyl)-2-methylthioadenosine (ms2iPA) at position 38. The methyl resonances of the latter two nucleosides are potential probes of the conformation of the anticodon loop, and of possible conformational changes during codon-anticodon binding and other tRNA functions.

We present here results of an NMR study of $tRNA^{tyr}_{coli}$, particularly the upfield region. Tentative identification of several resonances has been aided through comparison with the spectrum of $tRNA^{tyr}_{su^+_{III}}$, Form II (11). This molecule differs from wild type $tRNA^{tyr}_{coli}$ at two positions: the modified guanosine is changed to a C, and the ms2iPA lacks the thiomethyl group.

EXPERIMENTAL. Transfer RNA from E. coli was purchased from Plenum Scientific. 3.5 g of unfractionated tRNA were aminoacylated with tyrosine and fractionated on a 1000 ml benzoylated DEAE cellulose column (12). The tyrosine-$tRNA^{tyr}$ was eluted at about 10% ethanol in a 0-15% (v/v) gradient. These pooled fractions were further purified by chromotography on a smaller (200 ml) column of the same packing material, using a sharper ethanol gradient. Since no material ran at 10% ethanol in a similar column without aminoacylation of $tRNA^{tyr}$, the material was judged to be > 90% pure.

NMR spectra were taken on a Varian HR 220 spectrometer, using a C1024 time-averaging computer. Spectral peaks were referenced externally against either 3-(trimethylsilyl)-propanesulfonic acid (TSP) in D_2O, or undiluted hexamethyldisiloxane (HMS) in a concentric capillary.

All buffers for NMR were made up in D_2O, and the exchangeable tRNA protons were replaced with deuterium by repeated lyophilization and readdition of D_2O, 100.0 atom % D, Diaprep, Inc. The acetate buffer was made using deuterated acetic acid, 99.5% isotopic purity, Diaprep, Inc. Composition of buffers: A: 0.015 M $MgCl_2$, 0.015 M d_4-acetic acid, adjusted to pH 4.5 with NaOD, tRNA concentration about 25 mg/ml; B: 0.2M NaCl, 0.01M Na_2HPO_4, adjusted to pH 7 with NaOD, 0.02M $MgCl_2$, tRNA concentration between 20 and 25 mg/ml.

Transfer $RNA^{tyr}_{su^+_{III}}$, Form II, was prepared by a modification of the method of Gefter and Russell (11). We are most grateful to John Mack and Professor D. Srinivasan (Department of Biochemistry, College of Physicians and Surgeons, Columbia University) for generously supplying this t-RNA. For NMR studies the sample was dialyzed into phosphate buffer (B) and then lyophilyzed twice with readdition of D_2O.

The pH values reported are direct pH meter readings from a meter standardized with H_2O buffers.

RESULTS. The complete NMR spectrum of $tRNA^{tyr}_{coli}$ is shown in Fig. 1. Spectra of the upfield region at 28° and 43° and pH's 4.5 and 7.0 are given in Figure 2. Also shown in Figure 2 is a spectrum of $tRNA^{tyr}_{su^+_{III}}$, Form II, at pH 7.0, 28°. The major peaks visible in the upfield region are numbered in the

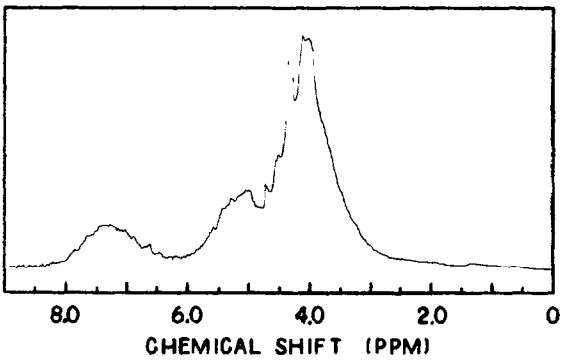

Fig. 1.

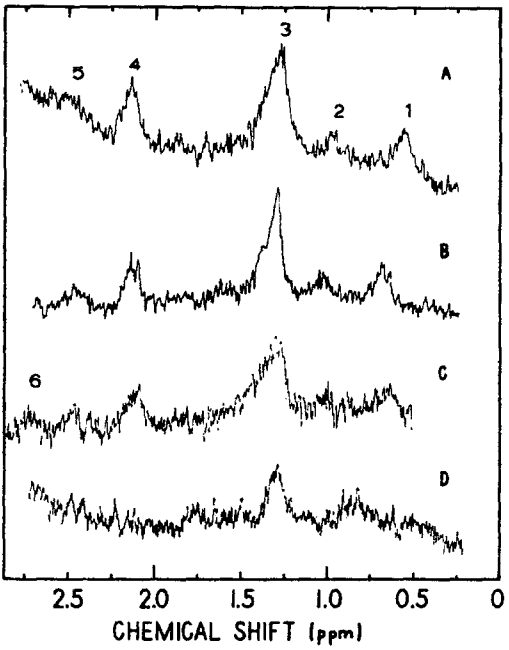

Fig. 2.

Figure 1. 220mHz spectrum of $tRNA^{tyr}_{coli}$; 25 mg/ml in D_2O, pH 4.5, 0.015M $MgCl_2$, 0.015M d_4-acetate, 28°. Chemical shifts are referenced relatives to the resonance of a capillary containing 5% TMS in CCl_4. 140 time-averaged scans. The broad resonance between 6.3 and 8.5 ppm is attributed to protons at position 8 of guanosine, positions 2 and 8 of adenosine, and position 6 of cytidine and uridine. About 2 ppm upfield of this region are the protons assigned to the 5-position in cytidine and uridine, and to the 1-position of the ribose ring. Near 4 ppm is a large group of resonances due to all the remaining ribose protons. The sharp peak at 4.4 ppm is residual HDO, distorted due to an artifact of the time averaged display.

Figure 2. 220MHz spectra of the high field region.

a. $tRNA^{tyr}_{coli}$; 25 mg/ml in D_2O, pH 4.5, 0.015 M $MgCl_2$, 0.015M d_4-acetate, 28°. 900 scans.
b. Sample of Fig. 2a except 43°. 900 scans.
c. $tRNA^{tyr}_{coli}$; 21 mg/ml in D_2O, pH 7.0, 0.02 M $MgCl_2$, 0.2M NaCl, 0.01 M Na_2HPO_4, 28°. 900 scans.
d. $tRNA^{tyr}_{su^+_{III}}$; ~20 mg/ml in D_2O, pH 7.0, 0.02M $MgCl_2$, 0.2M NaCl, 0.01 M Na_2HPO_4, 28°. 953 scans.

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figure, and their chemical shifts are summarized in Table 1.

Spectral data for the modified nucleosides of $tRNA^{tyr}$ are shown in Table II. Schweizer et.al. (16) have studied the effect of an isopentenyl

Table I. Chemical shift references are described in the experimental section. All values here have been corrected to a reference of 5% TMS (tetramethylsilane) in CCl_4, used in an external, coaxial capillary at 220MHz. Shifts are recorded in parts per million (ppm) of the applied field.

Peak#	Fig. 2, a	Fig. 2, b	Fig. 2, c	Fig. 2, d
1	1.55	0.69	0.65	
2	0.97	1.05	1.01	
3	1.28	1.33	1.32	1.3
4	2.12	2.18	2.12	
5		2.5	2.5	
6			2.7	

Table II. Selected Chemical Shifts of Nucleosides Found in $tRNA^{tyr}_{coli}$.

	δ[a] (ppm)	Solvent	Reference
ApiPA[b]			
$[=C(CH_3)_2]$	1.33	D_2O (pD 7.4 33°)	16
iPApA[c]			
$[=C(CH_3)_2]$	1.43	D_2O (pD 7.4 33°)	16
Ribothymidine			
$[CH_3]$	1.65	D_2O (pD 7.0 32°)	3
ms2iPA[d]			
$[=C(CH_3)_2]$	1.69 1.73	DMSO-D_2O	9
$[-SCH_3]$	2.55	DMSO-D_2O	9
2'-O-MeG			
$[-OCH_3]$	3.42-3.47[e]	DMSO-D_2O	5

a. Corrected to correspond to shift relative to external capillay of 5% TMS in CCl_4 at 220MHz.
b. Adenylyl $(3'\rightarrow5')$ N^6-(Δ^2-isopentenyl)adenosine.
c. N^6-(Δ^2-isopentenyl)adenylyl $(3'\rightarrow5')$ adenosine.
d. 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine.
e. Assigned in spectrum of $tRNA^{phe}_{yeast}$ in 83% DMSO.

group modification on the conformation of adenosine dinucleoside monophosphates. These results are also listed in Table II.

The spectrum of $tRNA^{tyr}_{su^+_{III}}$, Form II, shows only one distinct peak (at 1.3 ppm) in the high field region scanned. However, due to the small amount of sample available, the signal-to-noise ratio is quite poor. It seems clear, nonetheless, that peak 4 of $tRNA^{tyr}_{coli}$ is absent in the supressor tRNA spectrum, and peak 1 is either absent or greatly broadened.

DISCUSSION

Assignments. Peak 3 is assigned to the two isopentenyl methyl groups of ms2iPA. 1. Using the area under the resonance group between 6.3 and 8.5 ppm (Fig. 1) as a comparison, peak 3 is due to 5±1 protons. 2. The chemical shift is the same as that reported (16) for the methyl groups of ApiPA and is 0.1 ppm upfield of the iPApA methyl resonance. 3. Peak 3 appears in the spectrum of $tRNA^{tyr}_{su^+_{III}}$ Form II while the other numbered resonances either are not seen or are greatly altered.

Peak 4 is tentatively identified as the methylthio resonance of ms2iPA. 1. It does not appear in the spectrum of $tRNA^{tyr}_{su^+_{III}}$, Form II, which lacks the methylthio modifications. 2. Peak 4 is approximately 0.8 ppm downfield of peak 3 and, while ms2iPA has not been observed in D_2O solution, in DMSO the methylthio resonance appears 0.82 ppm downfield of the isopentenyl peaks (9).

Peak 1 may be due to a resonance from the Q base in the anticodon loop since the peak does not appear in the suppressor t-RNA spectrum. Its chemical shift suggests an aliphatic methyl group although other possibilities exist. Other resonances in the $tRNA^{tyr}_{coli}$ spectra may also be from the Q base. The methyl resonance of 2'-O-Me G is not expected to appear in the region scanned (5).

The ribothymidine methyl peak is apparently not resolved under the conditions used.

Interpretation of line widths. From their fluorescence depolarization studies using ethidium bromide intercalated in a helical region Tao et. al. (17) measured a rotational correlation time τ_D = 25nsec for $tRNA^{phe}_{yeast}$ and unfractionated tRNA in the presence of magnesium at 25°C. Their results also indicated that under these conditions the structure is only slightly elongated. We can, therefore, use τ_D to calculated approximate nuclear relaxation times on the basis of equations appropriate for isotropic reorientation.

For a methyl group having little freedom of internal motion ($\tau_{internal} >>$

τ_D), $1/T_2)_{calculated}$ = 400 sec^{-1} at 220MHz if intramolecular dipole-dipole relaxation dominates (18).

From Fig. 2a the observed relaxation rates are $1/T_2 \cong 75$ sec^{-1} for peak 4 and a maximum of 90 sec^{-1} for peak 3 assuming coincidence of the isopentenyl methyl resonances. These methyl groups must, therefore, experience significant internal rotation. (For a methyl group rotating rapidly on tRNA ($\tau_{internal} \ll \tau_D$) the predicted value of $1/T_2$ is 100 sec^{-1} (18).) Lack of fast internal rotation may be the explanation for why the ribothymidine methyl resonance is not resolved.

Effects of buffer and temperature changes. When the temperature is raised from 28° to 43° at pH 4.5, 0.015 M [Mg^{2+}], and low NaCl concentration, the peaks become somewhat narrower and better resolved as expected from faster overall rotation. However, no large structural changes are indicated. At 28° in a pH7 buffer containing 0.2M NaCl the peaks are somewhat broader than observed in the low salt buffer. This probably indicates some aggregation of the tRNA at the higher salt concentration. The effect is not large and the concentration of t-RNA (21 mg/ml) is apparently low enough to preclude extensive aggregation under these conditions. Some chemical shift changes are detected and we are currently investigating these more thoroughly under a variety of conditions including the addition of oligonucleotides.

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