NMR STUDY ON THE METHYL AND METHYLENE PROTON RESONANCES OF $tRNA^{Phe}_{yeast}$ *

L. S. Kan+ and P. O. P. Ts'o
Division of Biophysics
School of Hygiene and Public Health
The Johns Hopkins University
Baltimore, Maryland

and

F. v. d. Haar, M. Sprinzl and F. Cramer
Abteilung Chemie
Max-Planck-Institut
Göttingen, W. Germany

Received April 26,1974

Summary: The proton resonances of 13 methyl groups and 4 methylene groups belonging to 12 modified bases in $tRNA^{Phe}_{yeast}$ were investigated by 220 MHz NMR spectrometry. The chemical shifts and the linewidths at half height of these assigned resonances in tRNA were measured as a function of temperature from 21° to 80°C. The results indicate: (1) The anticodon loop does not associate with other components of the molecule and the side chain of the Y base protrudes out into the solvent; (2) The methyl groups $m^5C_{40,49}$, m^2G_{10}, and m^1A_{58} are not near any diamagnetic regions in the native tRNA; (3) The methyl and methylene groups in m^2_2G, T, and hU are magnetically shielded and immobilized to a great extent in the native conformation, implying that these bases are deeply involved in the tertiary structure of tRNA.

Nuclear magnetic resonance has been employed successfully to provide valuable information about the structure and conformation of tRNA's (1-6). This report concerns a nmr study on the methyl and methylene proton resonances of $tRNA^{Phe}_{yeast}$. The methyl resonances (from the modified bases), which are intense singlets consisting of three protons, and the methylene resonances (from C_5 and C_6 of hU), which are triplets, are well resolved at high temperature in the highfield region between the HDO signal and the tert-butanol signal. The

*This work was supported in part by NSF grant GB-30725X, NIH grant GM-16066-06, and a NATO research grant, No. 701. Experiments with the 220 MHz instrument were performed at the nmr Regional Facilities Center at the University of Pennsylvania, established by NIH research grant No. 1PO7RR-00542-01 from the Division of Research Facilities and Resources.
+NIH Postdoctoral Fellow, 1972-1974.

assignments of these methyl and methylene resonances from $tRNA^{Phe}_{yeast}$ will be published elsewhere; the assignments are based on a careful comparison of the resonances of the intact tRNA with the mononucleotides (or nucleosides) and appropriate oligonucleotide fragments, such as a 12-nucleotide fragment from the anticodon loop, ACmUGmAAYAψm^{5}CUG (7) or the TψCG (8), at varying temperature. As shown in the cloverleaf structure of $tRNA^{Phe}_{yeast}$ (fig. 1), the

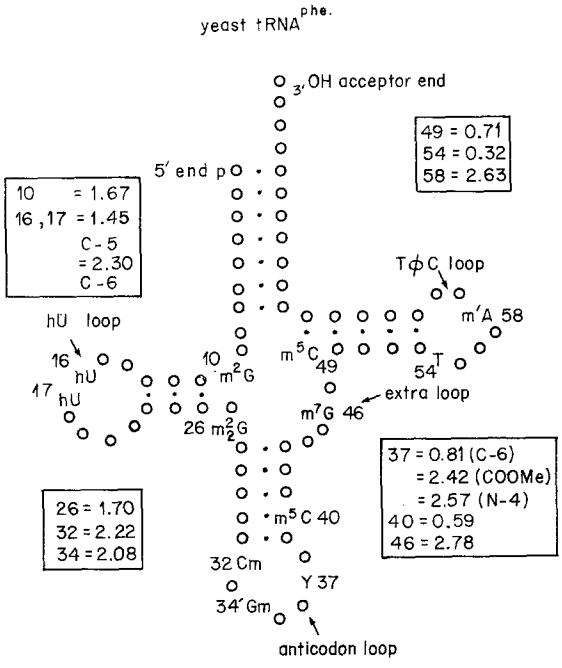

FIGURE 1: The cloverleaf structure of $tRNA^{Phe}_{yeast}$. The bases which show the highfield resonances are indicated. m^1A: N^1-methyladenosine; m^5C: 5-methylcytidine; Cm: 2'-O-methylcytidine; Gm: 2'-O-methylguanosine; m^2G: N^2-methylguanosine; m^2_2G: N^2,N^2-dimethylguanosine; m^7G: N^7-methylguanosine; T: 5-methyluridine; hU: 5,6-dihydrouridine; and Y: Y base. The chemical shift values of these proton resonances at 71°C are illustrated at four corners (in ppm referred to the methyl resonance of tert-butanol).

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modified nucleosides which contain various methyl and methylene groups are scattered throughout the molecule except the -CCA stem region. Important information about the environmental changes of these groups during the transition of tRNA conformation can be obtained from a nmr investigation.

Experimental: Highly purified $tRNA^{Phe}_{yeast}$ was obtained from bakers' yeast (9). After the final column chromatography step, the tRNA was desalted by passing

through a BioGel P2-column (3 x 20 cm) with water as eluant (9). The nmr spectrum of the tRNA was first measured at 23°C and then was heated to 80° in 6 hr for the collection of six spectra; the sample was then cooled to 21°C in 7 hr for the collection of seven spectra. The solution was reheated again to 54°C for additional collection of spectra. During this cycle of heating and cooling for 14 hr, all spectral changes were found to be completely reversible. The chemical shifts reported here refer to the internal standard, tert-butanol which is downfield to DSS by 1.24 ppm at 0° or 1.23 ppm at 80°C.

Results: The methyl and methylene resonances from $tRNA^{Phe}_{yeast}$ at three temperatures are shown in fig. 2. At 71°C, all signals are well resolved and have been assigned (fig. 2a). At lower temperatures (fig. 2b and 2c), most of these

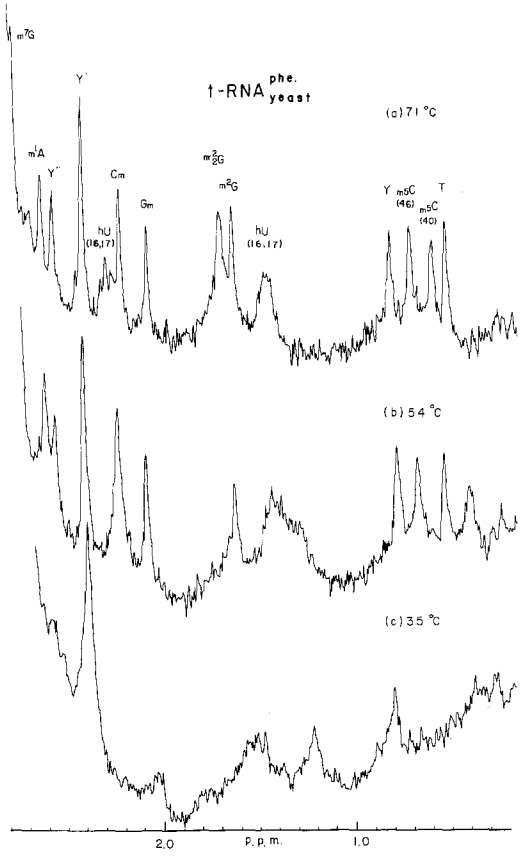

FIGURE 2: The nmr spectrum of the highfield proton resonance region of $tRNA^{Phe}_{yeast}$. The solution was prepared by dissolving 3.3 mg intact tRNA in 0.33 ml, 0.01 M potassium phosphate buffer in D_2O, pD = 7.0. No extra salt or magnesium ion was added. A Varian HR-220 nmr spectrometer equipped with a FT system was used. At 71°C or higher (a), all signals are well separated and assigned. Signal Y is the C_6-Me, Y' is COOOMe and Y'' is N_4-CH_3 on Y base. At 54°C (b), some peaks (T, m^2_2G and hU) are shifted to higher field and broadened. At 35°C (c), most peaks except Y' are very broad.

resonances become broadened and are shifted to a higher field. The responses of these signals to the temperature perturbation can be divided into three categories where: (1) The chemical shifts and the linewidths of the resonances are not sensitive to temperature. The two Y-COOMe groups (6 protons) belong to this category (fig. 3). (2) The chemical shifts of the

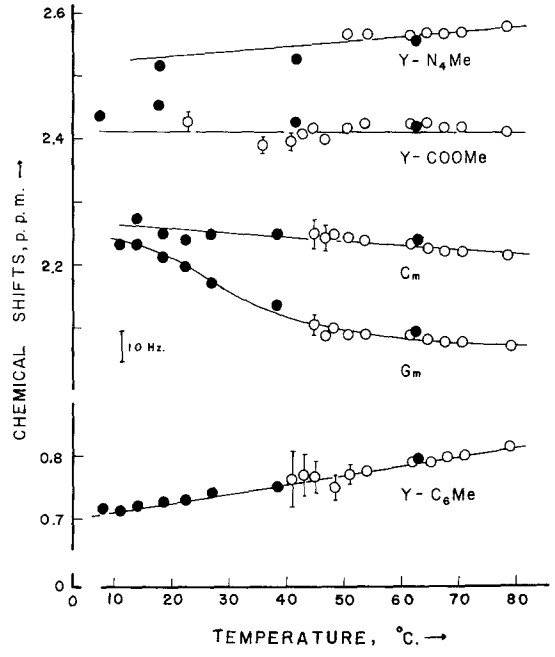

FIGURE 3: Plot of the chemical shift values vs. temperature of the methyl resonances from the anticodon loop both in $tRNA^{Phe}_{yeast}$ (open circles) and its isolated dodecamer fragment (filled circles). The linewidth at half height of these resonances are also indicated by the vertical bar. The circle has a width less than 3 Hz.

resonances in this category are slightly affected by the decrease in temperature (either shifting upfield or downfield, such as those shown in fig. 3), but the linewidths of these resonances are greatly broadened at temperatures below the transition. This category includes m^2G (the chemical shift of this methyl group is hardly affected by temperature, fig. 2), two m^5C, m^1A, C_6-CH_3, and N_4-CH_3 of Y, Gm, Cm, and perhaps m^7G. (Data on m^5C, m^1A and m^7G are not shown here.) Basically, the chemical shifts of the methyl groups in the

anticodon loop are not greatly affected during the thermal transition.

(3) The chemical shifts and the linewidths of the resonances in this category are both sensitive to temperature. Near the transition region, an abrupt upfield shift together with a large linewidth broadening of these resonances occurs with decreasing temperature. The methyl resonances of m_2^2G and T, as well as the methylene resonances of hU (C_5 or perhaps C_6), belong to this category (fig. 4). As reference, a uv melting curve of $tRNA^{Phe}_{yeast}$ measured at 259 nm is shown in fig. 5 with a transition midpoint, T_m, about 55°C. This

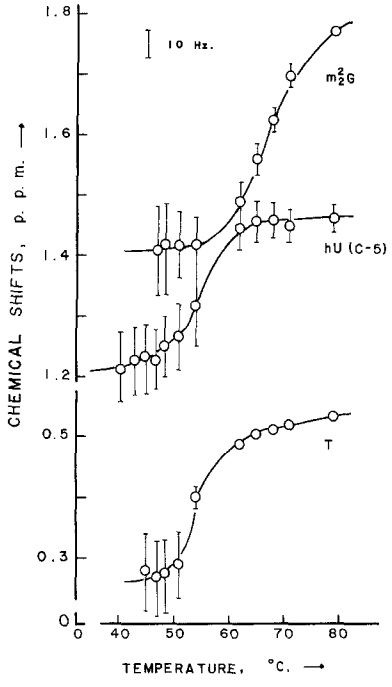

FIGURE 4: Plot of chemical shifts and linewidths at half height (denoted by vertical bar) vs. temperature for the methyl groups of T and m_2^2G; C_5 methylene group of hU. Both chemical shifts and linewidths show a cooperative transition. The T_m for hU and T is about 55° and for m_2^2G about 67°C.

measurement was made with the same tRNA preparation and the same solvent system as in the nmr studies, but with about 300-fold less concentration of tRNA.

Discussion: The above nmr results together with data from other laboratories

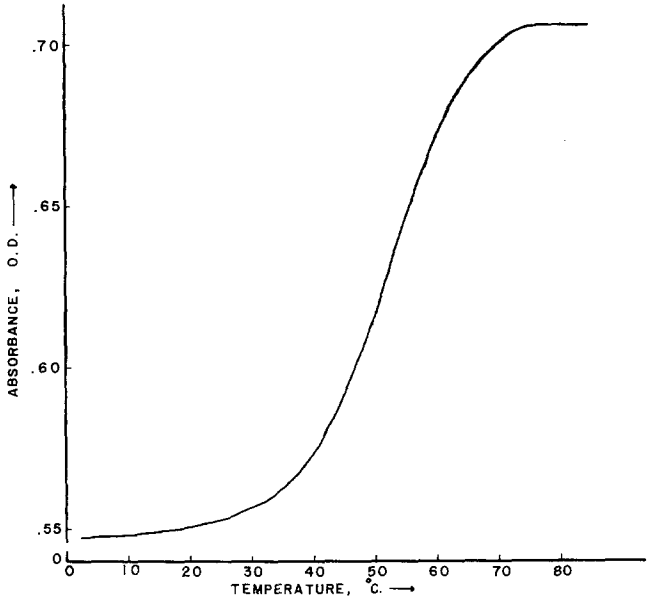

FIGURE 5: Absorbance vs. temperature profile of $tRNA^{Phe}_{yeast}$ at 259 nm. This sample is prepared by dissolving 0.55 A_{259} units of intact $tRNA^{Phe}_{yeast}$ in 1 ml D_2O solvent which contains 0.01 M phosphate and 0.02 M NaCl, pD = 7.0

(1,2,6) clearly indicate the highfield methyl and methylene resonances from the modified nucleosides can serve as useful probes for tRNA conformation in aqueous solution. The interpretation of the above data is based on the assumption that these spectral changes reflect intramolecular, conformational transition and not intermolecular aggregation. This assumption is justified since the investigation is carried out in relatively low salt solution (0.01 M) and in the absence of added Mg^{2+}; also dimer formation has never been described for $tRNA^{Phe}_{yeast}$. Another reason is that most of the spectral changes take place above 50°C (fig. 4) where the aggregation, if it exists, must be rather small.

The first major conclusion of this work is that the methyl groups in the anticodon loop of the intact $tRNA^{Phe}_{yeast}$ are in a similar magnetic environment as those in the isolated, single-stranded, dodecamer fragment (fig. 3). Most of these methyl resonances of the tRNA became broadened at temperatures below the transition (~45°C) as compared to the fragment, but the chemical shifts

are rather similar to each other. The Y-COOMe groups can be observed as a narrow line even at 10^{o}, suggesting a large rotational freedom of this group. The data suggest that the sidechain of the Y base protrudes out into the solvent and the anticodon loop does not associate with other components of the molecule.

The second conclusion is that the chemical shifts of the methyl resonances of m^5C and m^2G in the helical duplex, interestingly, are not very sensitive to temperature at the transition range (about 0.1 ppm from 45^{o}-80^{o}), even though the linewidths of these resonances are narrow at high temperature and are broad at low temperature (fig. 2). The linewidth data indicate the involvement of m^5C and m^2G in the conformational transition. This insensitivity of chemical shifts may be due to the locations of these methyl protons which are too far from the helical duplex to be influenced by the diamagnetic effect of the stacked bases. Similarly, the chemical shift, but not the linewidth, of the methyl group of m^1A in the TΨC loop is insensitive to temperature. All these results suggest that the methyl groups of these four bases are not near any diamagnetic regions in the native tRNA.

The third conclusion is based on the dramatic changes in both chemical shifts and linewidths of methyl and methylene groups of m_2^2G, T and hU at the transition region (fig. 4). The profile of the chemical shifts versus temperature indicate a cooperative phenomenon: The T_m^{nmr} on the curves of T and hU are nearly equal, i.e., around $55^{o}C$ with the transition starting at 48^{o} and ending at about $67^{o}C$. Though the T_m^{nmr} and the T_m^{uv} of these curves are similar (fig. 5), the transition of optical curve has a considerably wider range, starting from 40^{o} and ending about $72^{o}C$. The T_m^{nmr} of the m_2^2G curve is higher, about 67^{o}, and the transition does not start until 53^{o} and has not ended near $80^{o}C$. These four bases are not located in the helical, double-stranded region as predicted by the generally accepted cloverleaf model. Therefore, the nmr studies of the methyl and methylene protons may provide additional information about the conformational change of tRNA, especially in the non-basepaired regions.

These nmr results are consistent with the conclusions from x-ray diffraction studies on $tRNA^{Phe}_{yeast}$ crystal by Rich and coworkers (10). In their model, the anticodon loop is at one end of an "L" shaped molecule with the Y base sidechain protruding into the solvent. Also, in this model G_{15} is located close and may be hydrogen-bonded to C_{48}; and the dihU loop is close to the TψC loop and they probably interact with each other. The $m^2_2G_{26}$ is in the connecting region of the dihU stem with the anticodon stem. Clearly the methyl and the methylene groups of T, hU and m^2_2G are close to diamagnetic regions, since all these bases appear to be involved in the tertiary structure of the native $tRNA^{Phe}_{yeast}$.

Acknowledgement: We wish to express great appreciation to Dr. P. N. Borer for valuable discussion of this paper.

REFERENCES

1. K. M. Koehler and P. G. Schmidt, Biochem. Biophys. Res. Commun. 50, 370 (1973).
2. M. P. Schweizer, S. I. Chan, and J. E. Crawford, in Physico-Chemical Properties on Nucleic Acids (J. Duquesne, ed.), Vol. 2, Academic Press, London (1973).
3. J. E. Ladner and M. P. Schweizer, Nucleic Acids Res. 1, 183 (1974).
4. D. R. Kearns and R. G. Shulman, Acc. Chem. Res. 7, 33 (1974).
5. R. G. Shulman, C. W. Hilbers, Y. P. Wong, K. L. Wong, D. R. Lightfoot, B. R. Reid, and D. R. Kearns, Proc. Natl. Acad. Sci. (U.S.A.) 70, 2042 (1973).
6. I. C. P. Smith, T. Yamane, and R. G. Shulman, Canad. J. Biochem. 47, 480 (1969).
7. A. Maelicke, F. v. d. Haar, and F. Cramer, Biopolymers 12, 27 (1973).
8. D. Richter, V. A. Erdmann, and M. Sprinzl, Nature New Biol. 246, 132 (1973).
9. D. Schneider, R. Solfert, and F. v. d. Haar, Hoppe-seyler's Z. Physiol. Chem. 353, 1330 (1972).
10. F. L. Suddath, G. J. Quigley, A. McPherson, D. Senden, J. J. Kim, S. H. Kim, and A. Rich, Nature 248, 20 (1974).