NMR STUDIES OF ORGANIC SOLVENT DENATURED YEAST PHENYLALANYL TRANSFER RNA AT 220 MHZ[a]

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SUMMARY

Computer averaged proton magnetic resonance studies at 220 MHz of purified yeast $tRNA^{phe}$ denatured with dimethylsulfoxide have shown that protons of most of the modified nucleosides may be located and tentatively identified. The "melting" behavior of the modified nucleosides does not indicate that they are in more flexible regions of the tRNA secondary structure as it exists in water. Segments containing 5-methylcytidine and ribothymidine are most resistant to denaturation.

Introduction: Transfer RNA molecules contain many modified nucleosides (1, and references therein). With several exceptions, little is known concerning their importance in terms of tRNA structure and function. N^6-(Δ^2-isopentenyl)-adenosine or its 2-methylthio derivative have been shown to be essential for efficient binding of several aminoacyl tRNAs to ribosome-messenger complexes (2,3). The isoprene group was also shown to exert a structural influence at the dinucleoside monophosphate level by affecting the overlapping of base rings in adenylyl (3'→5')-N^6-(Δ^2-isopentenyl)adenosine compared with adenylyl (3'→5')adenosine (4).

NMR techniques are applicable in studies to explore the influence of modified nucleosides upon tRNA structure since the unique proton types of these unusual units may be identified, once the tRNA polymer has been rendered flexible through denaturation to lessen dipolar interaction between the individual groups. NMR studies have been done on bulk and fractionated tRNA

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denatured thermally (5,6), and by organic solvents (7). The solvent perturbation afforded narrow spectral lines for the individual nucleoside types as well as minimal chance for degradation. We report here NMR studies at 220 MHz of yeast phenylalanyl tRNA denatured by dimethylsulfoxide. Many of the modified nucleosides could be tentatively identified and followed through the denaturation process.

Experimental: Phenylalanyl tRNA was isolated by the BD cellulose method of Wimmer et al. (8) from bulk tRNA. Crude yeast synthetases were prepared by the procedure of Hoskinson and Khorana (9). Using the paper disc charging assay of Trupin et al. (10), the $tRNA^{phe}$ was 20-30 fold enriched in C^{14}-phenylalanine acceptance, depending upon the particular run. Thus the $tRNA^{phe}$ was 70-90% pure, and in a typical large-scale run, 300 mg $tRNA^{phe}$ was obtained from 15 g crude tRNA. The $tRNA^{phe}$ samples were converted to the lithium salts by passing through Dowex 50 (Li) columns for increased solubility in the mixed organic-aqueous solvent systems. Transfer RNA^{phe} minus the "Y" base next to the anticodon was obtained by the method of Theibe and Zachau (11). We thank the following for reference nucleosides: Dr. Jack Fox, Sloan-Kettering, ribothymidine; Dr. Arthur Broom, University of Utah, 1-methyladenosine; Dr. Roland Robins, ICN Nucleic Acid Research Institute, N-2-methylguanosine, N-2-dimethylguanosine and 2'-O-methylguanosine. β-Pseudouridine was recrystallized from an α,β-isomeric mixture with methanol (12). All other nucleosides were obtained commercially.

Results and Discussion: In Figure 1 is presented a single scan of phenylalanyl tRNA in 83% by volume DMSO-d_6. This well-resolved spectrum illustrates the denaturing influence of DMSO when compared with thermally denatured yeast alanyl tRNA (6). Region I contains adenine H-8 and H-2, guanine H-8 and pyrimidine H-6. In region II is found pyrimidine H-5 and H-1' of all units. Other ribose protons absorb between regions II and III. Methylene and methyl protons from modified nucleosides are found in regions III and IV. The sharp peak at δ1.35 ppm is of unknown origin. Resonances of varying intensities

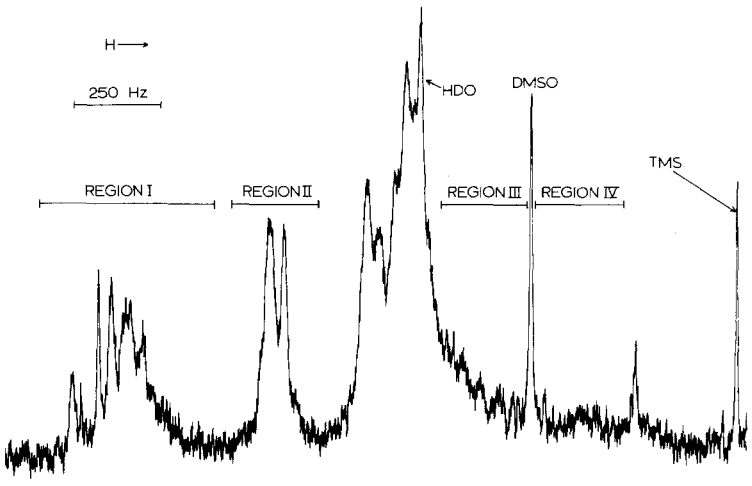

Figure 1. Single scan spectrum of $tRNA^{phe}$ (100 mg/ml) in 83% v/v DMSO-d_6/D_2O. All nmr samples were lyophilized at least twice from D_2O, and were prepared and sealed under vacuum using 100.0 atom % d D_2O and 100.0 atom % d DMSO-d_6 from Diaprep, Inc. PMR spectra were taken on a Varian HR-220 spectrometer. The sample temperature was controlled to $\pm$ 1°C (ethylene glycol splitting) by a Varian V-4343 variable temperature unit. Time-averaged spectra were taken using either Fabritek 1074 or Varian C-1024 computers. A Hewlett-Packard 200 ABR audio oscillator was used to produce frequency side bands for triggering the computer when field sweep spectra were taken. The frequency of the sideband was counted with a Hewlett-Packard 5221B electronic counter. Chemical shifts were referenced to a capillary containing a dilute solution of TMS in carbon tetrachloride.

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were found in this position in nearly all samples examined, including the crude tRNA, and in purified tRNAs of *E. coli* (13). Passage of the tRNA through Sephadex G-200 columns in 7 M urea did not remove this material, which may be due to tightly bound peptides.

Time averaged spectra of the various regions are illustrated in Figures 2 and 3 as a function of DMSO addition. The marked denaturing effect of DMSO is readily noted, both from standpoints of chemical shifts and line widths. Movement of chemical shifts to lower field is characteristic of disruption of base stacking by DMSO (14). The dramatic line narrowing induced by DMSO results as the tRNA becomes a flexible polymer. This denatured form is presumably attained partially as a result of the DMSO induced breakup of water structure (15) which assists in maintenance of the ordered biopolymer and is

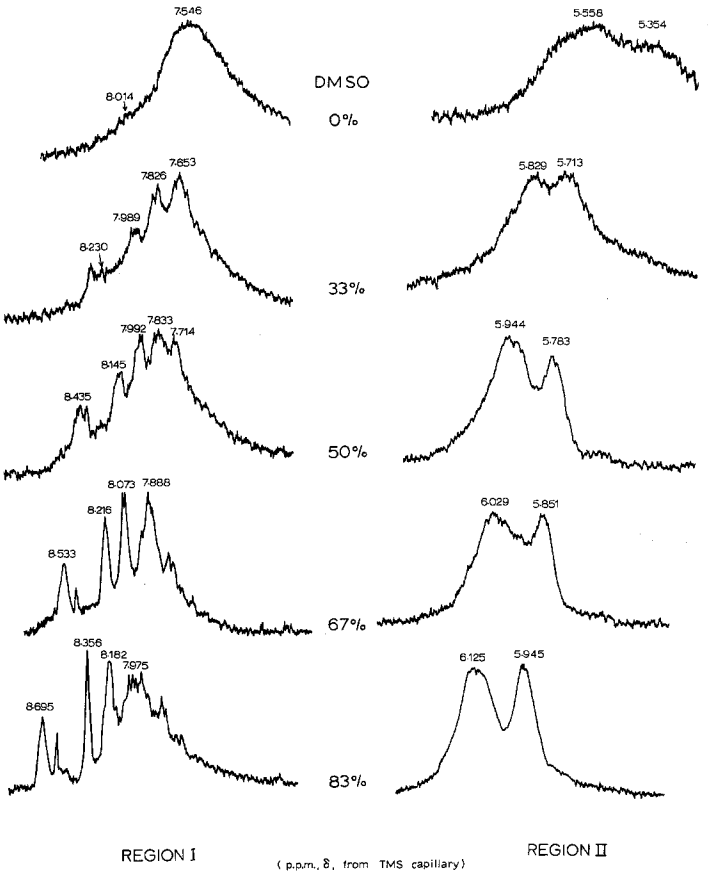

Figure 2. Time averaged spectra (10-30 scans) of regions I and II as a function of DMSO denaturation of $tRNA^{phe}$. Peak assignments in the spectra of the 83% DMSO samples were based upon comparisons of chemical shifts with the reference nucleosides in the same solvent. Region I: adenosine H-8 (8.695); adenosine H-2 (8.356); guanosine H-8 (8.182); pyrimidine H-6 (7.975). The sharp spike at 8.58 as well as the peaks at 7.84 and 7.77 ppm are not assigned. These resonances were not present in all $tRNA^{phe}$ samples examined. However, a small peak always occurs about 7.82 ppm which may be the H-6 of pseudouridine. The splitting of the pyrimidine H-6 peak at 7.975 probably is an artifact of time averaging. Region II: H-1' of all residues (6.125); pyrimidine H-5 (5.945).

partially due to DMSO hydrogen bond acceptor interaction with the bases (16). As may be best noted in region I, Figure 2, the greatest change in the spectrum occurs between 50 - 60% DMSO, although a gradual line sharpening is visible from 0 - 50% DMSO. This behavior is confirmed by optical rotation studies (17) of $tRNA^{phe}$ as a function of addition of DMSO and other organic sol-

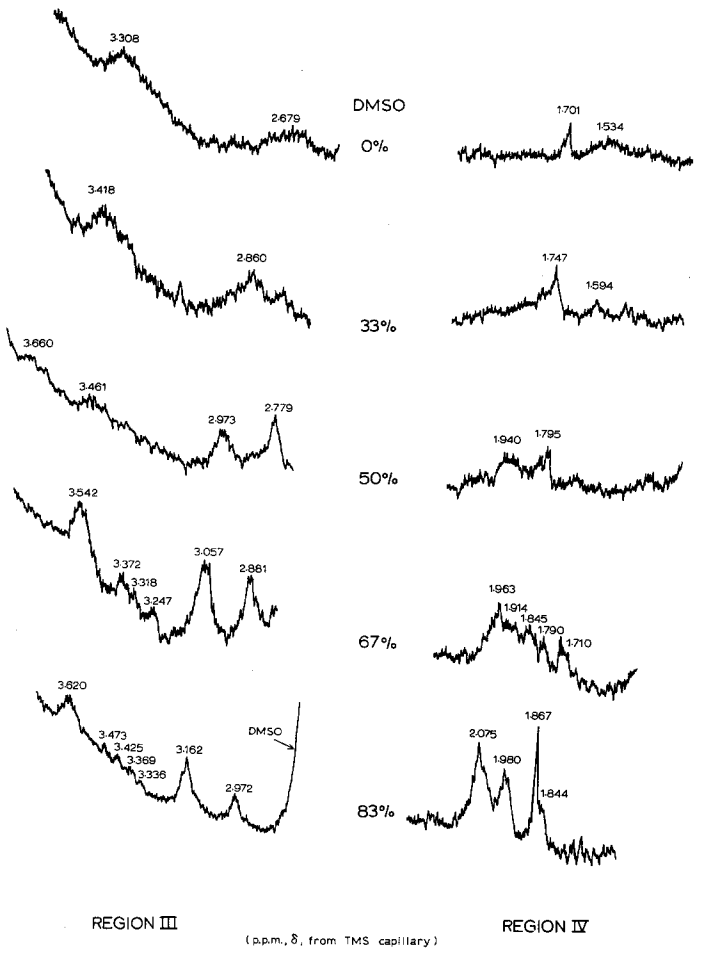

Figure 3. Time averaged spectra (40-80 scans) of regions III and IV as a function of DMSO denaturation of $tRNA^{Phe}$. Peak assignments in the spectra of the 83% samples (Region III): dihydrouridine H-5's (3.620); $-CH_3$ of 2'-O-methylguanosine and 2'-O-methylcytidine (3.473, 3.425); $-CH_3$'s of N-2-dimethylguanosine (3.162); $-CH_3$ of N-2-methylguanosine (2.972). In the spectrum at 50% DMSO the peak at 3.660 ppm may be due to the $-CH_3$ of 7-methylguanosine. Region IV: $-CH_3$'s of 5-methylcytidine (2.075); $-CH_3$ of ribothymidine (1.980) The sharp spikes at 1.701, 1.747 and 1.867 respectively, in the 0, 33 and 83% DMSO spectra are probably artifacts.

vents and indicates a continuous denaturation, with a significant transition occurring with a midpoint at about 55% DMSO.

Most of the resonance lines in the 83% DMSO spectra of Figures 2 and 3 were tentatively assigned by comparison with nucleoside chemical shifts in this solvent system and upon deuteration at C-8 of purines (see captions of

Figures 2 and 3). In the primary structure of yeast $tRNA^{phe}$ (18) the "Y" nucleoside is found at the 3'-end of the anticodon. A recent publication reports the structure of the "Y" base (19). This highly substituted guanine base has several $-CH_2$ and $-CH_3$ groups which would be expected to appear in regions III and IV. Since we did not have any "Y" nucleoside as reference, we compared $tRNA^{phe}$ before and after treatment with dilute HCl, which cleaves the "Y" base from the tRNA (11). However, broad spectral patterns were observed for the $tRNA^{phe}_{HCl}$ in 83% DMSO which prevented any assignment of $tRNA^{phe}$ resonances to the "Y" base. We have compared $tRNA^{phe}$ and $tRNA^{phe}_{HCl}$ in studies involving solvent and thermal deuteration (17) and find that the solution conformation of $tRNA^{phe}_{HCl}$ has been altered, with a reduced extent of "melting" by solvents such as DMSO and ethylene glycol. The ability to accept C^{14}-phenylalanine is not impaired, in agreement with the results of Thiebe and Zachau (11).

Comparisons of spectra from the 33% and 83% DMSO samples show that line widths of protons such as adenosine H-2 and guanosine H-8 decrease from ~ 50 Hz to 8-10 Hz, whereas those such as $-CH_3$ of N-2-dimethyladenosine, $-CH_2$ of dihydrouridine and $-CH_3$ of 5-methylcytidine decrease from 20-25 Hz to 10-14 Hz. This differential behavior at first glance would be interpreted as evidence for the location of the modified nucleosides in more flexible portions of the tRNA in whatever ordered structure is assumed in water[e]. One must remember, however, that the adenosine and guanosine resonances represent an envelope of many peaks due to individual residues and that these resonance positions become more nearly equivalent in the flexible polymer existing in the high DMSO containing solution, thus the envelope itself becomes narrower. We cannot determine, therefore, that the modified nucleosides are in more

[e] tRNA in aqueous solution has a Tm ~ 10°C lower than aqueous solutions of tRNA in the presence of about 0.1 M NaCl; this latter solution condition presumably is necessary for the cloverleaf structure to exist (20). No NaCl was used in the present study since it is not soluble in the mixed DMSO-water systems.

flexible regions in the tRNA secondary structure as it exists in water. In fact, it appears that segments containing 5-methylcytidine and ribothymidine are the most rigid, since marked line narrowing occurs after 67% DMSO (Figure 3, region IV) whereas all the other bases have been mobilized at 50-67%. It is of interest to note that E. coli $tRNA^{fMet}$ in 83% DMSO (13) is not as flexible as yeast $tRNA^{phe}$ which may reflect the higher G-C content, thus the greater stability of the former to solvent denaturation (21). Other purified tRNAs are currently under examination.

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