PMR STUDIES OF ORGANIC SOLVENT DENATURED TRANSFER RNA

Martin P. Schweizer

Department of Experimental Therapeutics, Roswell Park Memorial Institute
Buffalo, New York 14203

Received July 1, 1969

SUMMARY

Unfractionated transfer RNA from yeast, E. coli B and rat liver denature in dimethylsulfoxide to flexible macromolecules which display proton magnetic resonance spectra resolvable into individual nucleoside classes. Potentially this method provides a means of obtaining information regarding the role of modified nucleosides in tRNA structure.

INTRODUCTION

Numerous modified nucleosides have been found in transfer RNA (tRNA). These include various methylated compounds (1-4), pseudouridine (5), dihydrouridine (6), thiolated pyrimidine derivatives (7), N^6-(Δ^2-isopentenyl)adenosine (8) and its 2-methylthio derivative (9), and N-[9-(β-D-ribofuranosyl)-purin-6-ylcarbamoyl] threonine (10, 11). Elucidation of tRNA primary sequences is progressing rapidly (12). When the sequences are arranged to maximize base pairing and helical structure, the cloverleaf model of tRNA secondary structure emerges. Undoubtedly the modified nucleosides play an important role in tRNA secondary structure because of their potential influence in altering base pairing and base-stacking. Thus the substituted nucleosides are thought to indicate where loop regions exist and are usually considered to reside within such presumably flexible loops.

We have initiated a program to more fully explore the relationship between nature, location and content of modified nucleosides and tRNA structure and function. NMR techniques are applicable since the unique proton types of the modified nucleosides may be identified, provided the tRNA has been rendered flexible through

denaturation to lessen dipolar interaction between individual units. Thermal denaturation of tRNA has been studied by NMR (13-15). Most succinctly, the 220 MHz study by Smith et al. (15) on yeast alanyl tRNA showed that certain protons of modified bases could be tentatively identified, although the resonance lines were still quite broad.

Because there is minimal opportunity for degradation, the use of organic solvents as denaturing agents is preferable. Formamide and dimethylsulfoxide reversibly denature nucleic acids (16). Dimethylsulfoxide (DMSO) has also been shown to disrupt stacking interactions in mono-and dinucleotides (17) and polynucleotide complexes (18). We report here preliminary results of NMR investigations of DMSO denatured tRNA.

EXPERIMENTAL

Unfractionated yeast tRNA was prepared according to the method of Holley (19). E. coli B tRNA was purchased from Schwarz Bioresearch and General Biochemicals, Inc. supplied rat liver tRNA. Deuterium oxide (99.8%) was from Mallinkrodt and deuterated dimethylsulfoxide (99.5%) from Diaprep, Inc., Atlanta, Ga. or Mallinkrodt. Samples of tRNA for NMR analysis were generally 35 mg by weight in 0.5 ml solvent. Chemical shifts of various tRNA resonances varied 0.03-0.05 ppm with 30% changes in concentration in the solvent mixtures. Spectra were obtained with a Varian A-60A spectrometer equipped with a C-1024 computer. Ambient probe temperatures were 33-35° (ethylene glycol splitting). Chemical shifts were measured from an external tetramethylsilane capillary. No bulk susceptibility corrections were made.

RESULTS AND DISCUSSION

The data exhibited in Figure 1 clearly shows the denaturing action of DMSO. In spectrum (a), unfractionated yeast tRNA in D_2O containing 1 M NaCl displays very broad lines, as has been reported by others (13-15). Solution viscosity is not important here, since the HDO line widths are 1-2 Hz. Between 7.5 and 9.2 ppm (Region I), the following protons absorb: H-8 and H-2 of adenine, H-8 of guanine, H-6 of cytosine and uracil residues. Region II (5.0-7.5 ppm) contains H-5 protons of cytosine and uracil residues and H-1' of all nucleoside units. In spectrum (a)

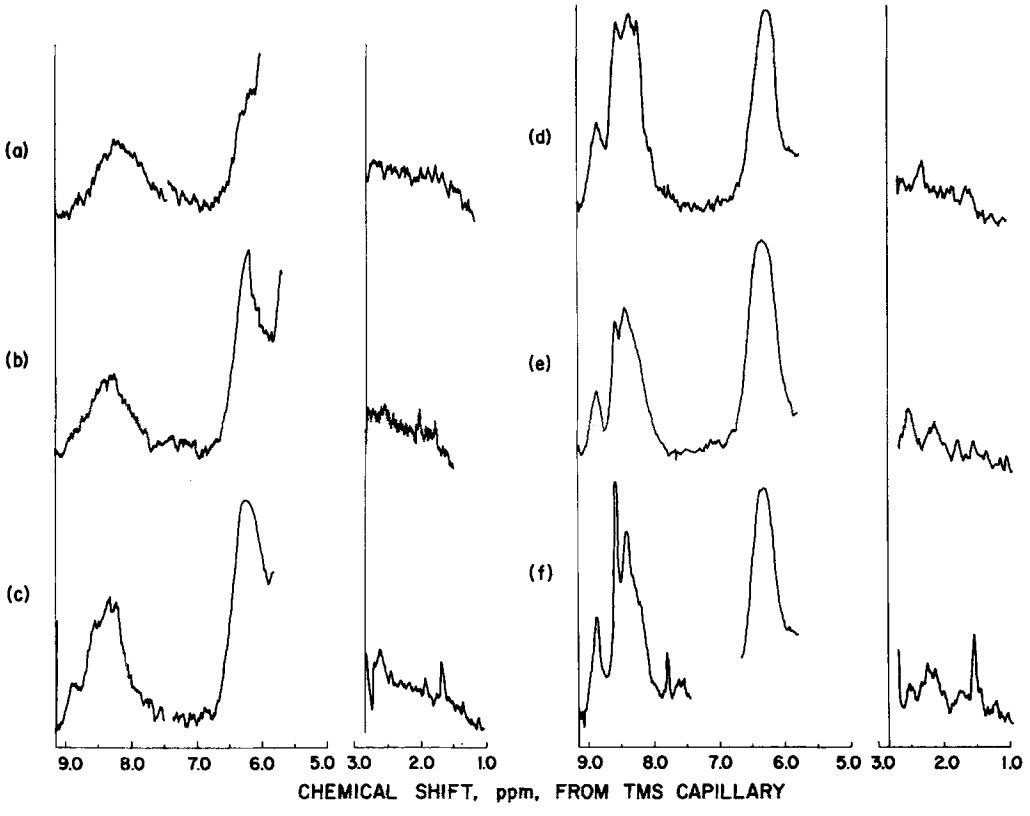

Figure 1

Effect of DMSO on yeast tRNA spectrum. All samples 35 mg/0.5 ml solvent; volume ratios of DMSO to D_2O are given, followed by the number of repetitive scans on the C-1024 for each of the three chemical shift regions; 9.2-7.5 ppm (I), 7.5-5 ppm (II) and 3-1 ppm (III). (a) 1 M NaCl in D_2O [I, II, III (100)]; (b) 25:75 [I (90), II, III (100)]; (c) 50:50 [I, II, III (100)]; (d) 55:45 [I (100), II (49), III (100)]; (e) 70:30 [I (64), II (49), III (100)]; (f) 85:15 [I (69), II (49), III (100)].

---

this resonance pattern is partially obscured by HDO. C-methyl protons would absorb in Region III, 1-3 ppm. Because of the residual protonated DMSO it was not possible to scan the chemical shift region where N-methyl and dihydrouridine methylene protons absorb.

Transfer RNA forms aggregates in 1 M NaCl, thus the combined line widths in spectrum (a) have contributions from this association as well as tRNA secondary structure. Comparisons of (a) with tRNA in D_2O containing no added salt show 15%

narrower Region I and II combined resonances in the latter, a similar result to that obtained by Smith et al. (14). No salt was added to any of the solutions containing DMSO.

In a solution containing 25% v/v DMSO-d_6, Region II now is visible [Figure 1 (b)], band width being 25-30 Hz, partially due to removal of overlap from adjacent HDO. The band shape of Region II remains essentially the same from this point through solutions containing 85-95% DMSO.

Considerable fine structure emerges in I for the tRNA in 50:50 DMSO:D_2O [Figure 1 (c)]. Definite peaks are observed at δ 8.85, 8.58, 8.38 and 8.25 ppm. With the exception of the resonance at 8.25 ppm, these lines become more prominent with DMSO addition. The 8.25 ppm peak merges with that at 8.40 ppm in the 70 and 85% DMSO solutions [Figure 1 (d) and (e), respectively].

All resonances shift downfield 0.04-0.06 ppm with increase in DMSO content, indicating loss of diamagnetic interaction among neighboring bases (17 and references therein). The change in bulk susceptibility with DMSO addition results in upfield shifts [about 0.2 ppm from D_2O to pure DMSO (18)].

Because purine H-8 protons may be exchanged with deuterium (13,20), it was possible to assign these protons in transfer RNA. In Figure 2 is displayed Region I for three solutions of yeast tRNA. The top portion is 85:15 DMSO:D_2O, the center 95:5 DMSO:D_2O. More heating on the steam bath was required to dissolve tRNA in 95% DMSO solution, consequently it may be observed that the areas of resonances at 8.89 and 8.44 are decreased with respect to that at 8.60 ppm. Nearly complete deuteration at C-8 of adenine and guanine is obtained, as shown in the bottom spectrum of Figure 2, by heating 2 hrs at 90° in D_2O, lyophilizing and redissolving the tRNA in 95:5 DMSO:D_2O. On the basis of this data and comparison with various mononucleotides (21), it is possible to arrive at the following assignments: adenine H-8, δ 8.85-8.90 ppm; adenine H-2, δ 8.57-8.63; guanine H-8, δ 8.38-8.45; pyrimidine H-6, δ8.25-8.30; pyrimidine H-5 and H-1' of all residue δ 6.25-6.37.

That partial deuteration has occurred in spectrum (f), Figure 1, may be seen through comparison of peak areas with (e). Some heating of the 85:15 DMSO:

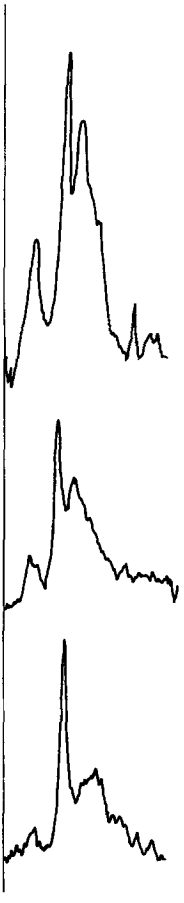

Figure 2

Partial deuteration at C-8 of adenine and guanine residues of yeast tRNA. Region I of the following samples are shown: 85:15 DMSO:D_2O (top, 69 scans); 95:5 DMSO:D_2O, heated on steam bath until tRNA dissolved (center, 49 scans); tRNA dissolved in D_2O, heated 2 hrs at 90°, lyophilized and redissolved in 95:5 DMSO: D_2O (bottom, 49 scans).

D_2O sample was required, but none for the 70:30 solution. It is evident that H-8 of guanine (δ 8.44) is exchanged more rapidly than H-8 of adenine (δ 8.89) from this comparison, in agreement with McDonald & Phillips (13).

The respective half-height line widths ($\Delta\nu$ 1/2, Hz) in spectrum (f), Figure 1 are: ^{A}H-8 (9), ^{A}H-2 (6), ^{G}H-8 (10). These values, while much larger than those for monomers (1-2 Hz), are composites of all the member nucleosides of the various types and are in turn considerably less than those found for thermally

"melted" alanine tRNA (15). Thus tRNA in DMSO presumably is more flexible than the heat denatured material. Ts'o et al. (22) reached similar conclusions in the case of formamide denatured nucleic acids and polynucleotides.

Unfortunately, residual protonated DMSO obscures part of the methyl region, particularly those methyl groups attached to nitrogen. However, the C-methyl region [ribothymidine (δ 2.24), 5-methylcytidine (δ2.32)] may be examined. Tentatively, [Figure 1 (d-f)], it is possible to observe broad peaks in the δ2.2-2.4 ppm area where these methyl protons absorb. The origin of the sharp peak at δ1.6 ppm in (f) is not known; it is possibly the high-field member of the C^{13}-H doublet from DMSO. Further elucidation of the methyl regions await studies to be reported using instrumentation at higher frequency, other deuterated denaturing solvents, and amino acid specific tRNA species.

It is of interest to note that comparisons of unfractionated tRNA of yeast, E. coli B, rat liver, all in 70 : 30 DMSO:D_2O show qualitative differences. For example, the E. coli B tRNA may be more extensively denatured than yeast tRNA. The Region I spectrum of the E. coli B sample in 70 : 30 DMSO:D_2O is quite similar to that of the yeast tRNA in 85% DMSO. On the contrary, rat liver tRNA appears to be less denatured than that of yeast, the Region I spectrum of the rat liver tRNA in 70% DMSO being similar to the 50% DMSO solution of the yeast sample. A consideration which prevents quantitative comparisons of these samples of bulk tRNA from different organisms is that the respective solution viscosities increase in the order E. coli < yeast < rat liver. This increase is monitored by the HDO line widths, which are, respectively, 2.5, 3.5 and 5.3 Hz. Thus the tRNA lines may be partially broadened due to spin-lattice interaction.

This study has demonstrated that tRNA in DMSO-D_2O solution is more flexible than heat denatured material, enabling more facile recognition of the individual nucleoside classes. Thus it seems possible that some information may be gained in the future concerning the importance of minor nucleosides in tRNA secondary structure. If the modified nucleosides are located in loop regions, which may be more flexible, the methyl resonances should appear earlier, since

the loop regions should denature fastest.

ACKNOWLEDGMENT

The author would like to thank Drs. Walter Korytnyk and Moshe Neeman for providing the nmr equipment. This work was supported by USPHS grant No. CA-10835.

REFERENCES

1. D.B. Dunn, Biochim. Biophys. Acta, 34, 286 (1959).
2. P.L. Berquist and R.E.F. Mathews, Biochem. J., 85, 305 (1962).
3. R.H. Hall, Biochemistry, 3, 876 (1964).
4. P.R. Srinivasan and E. Borek, Science, 145, 548 (1964).
5. D.B. Dunn, J.D. Smith and P.F. Spahr, J. Mol. Biol., 2, 113 (1960).
6. R. Holley, J. Apgar, G.A. Everett, J.T. Madison, M. Marquisee, S.H. Merrill, J.R. Penswick and A. Zamir, Science, 147, 1462 (1965).
7. M. Lipsett, J. Biol. Chem., 240, 3975 (1965).
8. M.J. Robins, R.H. Hall, R. Thedford, Biochemistry, 6, 1837 (1967).
9. J. Burrows, D.J. Armstrong, F. Skoog, S.M. Hecht, J.T.A. Boyle, N.J. Leonard and J. Occolowitz, Science, 161, 691 (1968).
10. G.B. Chheda, R.H. Hall, D.I. Magrath, J. Mozejko, M.P. Schweizer, L. Stasiuk and P.R. Taylor, Biochemistry, in press.
11. M.P. Schweizer, G.B. Chheda, L. Baczynskyj and R.H. Hall, Biochemistry, in press.
12. J.T. Madison, Ann. Rev. Biochem., 37, 131 (1968).
13. C.C. McDonald and W.D. Phillips, Biopolymers, 3, 609 (1965).
14. I.C.P. Smith, T. Yamane and R.G. Shulman, Science, 159, 1360 (1968).
15. I.C.P. Smith, T. Yamane and R.G. Shulman, Can. J. Biochem., 47, 480 (1969).
16. G.K. Helmkamp and P.O.P. Ts'o, J. Am. Amer. Soc., 83, 138 (1961).
17. P.O.P. Ts'o, N.S. Kondo, M.P. Schweizer and D.P. Hollis, Biochemistry, 8, 997 (1969).
18. M.P. Schweizer, Ph.D. Thesis, The Johns Hopkins University, Baltimore, Md. (1968).
19. R.W. Holley, Biochim. Biophys. Acta, 55, 374 (1962).
20. F.J. Bullock and O. Jardetsky, J. Org. Chem., 29, 1988 (1964).
21. M.P. Schweizer, A.D. Broom, P.O.P. Ts'o and D.P. Hollis, J. Am. Chem. Soc., 90, 1042 (1968).
22. P.O.P. Ts'o, G.K. Helmkamp and C. Sander, Biochim. Biophys. Acta, 55, 584 (1962).