300 MHz PMR STUDIES ON THE CONFORMATION OF THE HEXANUCLEOTIDE, 2'OMeGpApApYpApψ, FROM THE ANTICODON LOOP OF TORULA YEAST $tRNA^{phe}$

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SUMMARY

A complete temperature study of the proton resonances of the hexanucleotide, 2'-OMeGpApApYpApψ, from Torula yeast $tRNA^{phe}$ has been carried out at 300 MHz. The data has been interpreted in terms of a base stacked oligomer in which the glycosyl conformation of the Y-nucleoside changes from syn to anti with temperature increase. An alternative structure for the Y-base is proposed to permit this conformational change.

INTRODUCTION

Among the numerous modified nucleosides found in transfer RNA (1), a class of highly substituted guanine derivatives, the so-called Y-bases, has been the subject of considerable research. Located at the 3'-end of the anticodon in $tRNA^{phe}$ from many tissues, the structure of the Y-base has been reported in the case of baker's yeast (2), torula yeast (3; "Yt") and bovine liver (4). Excision of Y-base from $tRNA^{phe}$ by treatment with HCl (5) results in altered structural (6) and functional (7) properties.

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We have employed NMR techniques in an effort to gain information about the involvement of modified nucleosides in the structure and conformation of tRNA (8,9) and related oligonucleotides (10). In the case of yeast $tRNA^{phe}$ (8) it was not possible to monitor the Y-base effectively because of broad-spectral line associated with the polymer, thus we report here 300 MHz pmr studies of a hexanucleotide, 2'-OMeGpApApYpApψ, isolated from the anticodon loop of torula yeast $tRNA^{phe}$. A recent absorbance and flourescence study (11) of a similar hexamer (and other oligonucleotides) from the anticodon region of baker's yeast $tRNA^{phe}$ has shown that Y-base is intimately involved in the base-stacked structure. 300 MHz pmr measurements afford the opportunity to monitor each residue in the oligonucleotide and thus to obtain a multifaceted probe of the conformation and the changes at each site as a function of some external perturbation such as change of temperature.

EXPERIMENTAL

Torula yeast tRNA obtained from Sanyo Co., Tokyo was treated by the method of Avital and Elson (12) to remove ribosomal RNA. $tRNA^{phe}$, 6-fold enriched in phenylalanine acceptance, was obtained by modifications of the Tener method (13). The material of interest was eluted from BD cellulose columns between 0.9 and 1.2 M NaCl in a 0.5-2 M gradient. 900 mg of the partially purified $tRNA^{phe}$ was digested with T_1 RNase using a linear scale-up of the procedure of RajBhandary, et al. (14). Products of the digestion were separated on a 2 x 80 cm DEAE (C1) column using a 0.0-0.3 M NaCl gradient in 0.02 M Tris HCl, pH7.3 containing 7 M urea. The desired oligonucleotide, presumably the dodeca- or tridecanucleotide, was identified by fluorescence emission at 438 nm. Degradion of 1700 A_{260} of this material was carried out according to the method of RajBhandary, et al. (15) using pancreatic RNase and alkaline phosphatase. The flourescent compound was separated from the digestion mixture using DEAE chromatography as mentioned above. Finally, the hexamer was separated from non-fluorescent oligonucleotides by chromatography

on DE-52 (2 x 30 cm) using 0.0-0.3 M NaCl in 0.01 M phosphate buffer, pH 7.0. 50 A_{260} of the hexamer was obtained. This material was homogeneous in paper chromatography.

RESULTS AND DISCUSSION

The uv profile of the purified oligonucleotide consisted of transitions at λmax=258 nm and 244 nm with nearly equal extinctions. A characteristic fluorescence emission was observed at 420 nm upon excitation at 265 nm. These properties are similar to those reported by Maelicke, et al. (11) for a hexanucleotide, 2'-OMeGpApApYpApψ, excised from tRNA of baker's yeast. Torula $tRNA^{phe}$ is homologous in sequence with baker's $tRNA^{phe}$ for this region of the anticodon loop (16). The only difference in the torula derived hexanucleotide lies in the nature of the Y-base, which is a less complicated guanine derivative (3; see I, fig. 1) than found in baker's yeast (2).

A typical 300 MHz-FT spectrum is shown in fig. 1(A) with an expansion of the aromatic region in (B). The downfield peak at -5.23 ppm arises from some impurity in the sample and has a higher intensity and a much longer relaxation time compared to the other downfield peaks. Two additional small upfield peaks at 0.59 ppm and 1.67 ppm are probably also due to impurities. The ten peaks in the aromatic region and the three upfield methyl peaks are features consistent with a hexanucleotide, 2'-OMeGpApApYpApψ, in which the Y-base has both $N\text{-}CH_3$ and $C\text{-}CH_3$ substituents as proposed by Kasai, et al. (3). The assignment of the methyl resonance at δ-0.52 ppm to the $N\text{-}CH_3$ of Y and the peak at δ-0.21 ppm to the $2'\text{-}O\text{-}CH_3$ of G was based upon the observation that upon heating for 2 hours at 77°C at pH 5, the Y-base is 50% cleaved, leading to two sets of peaks at -0.52 and +1.0 ppm, one set due to the free Y-base.

In figure 2 is presented the aromatic proton chemical shift dependence upon temperature. In considering the temperature effects with the aid of space filling molecular models, the hexamer was assumed to adopt a right-handed helical conformation with the individual monomer moieties generally in the

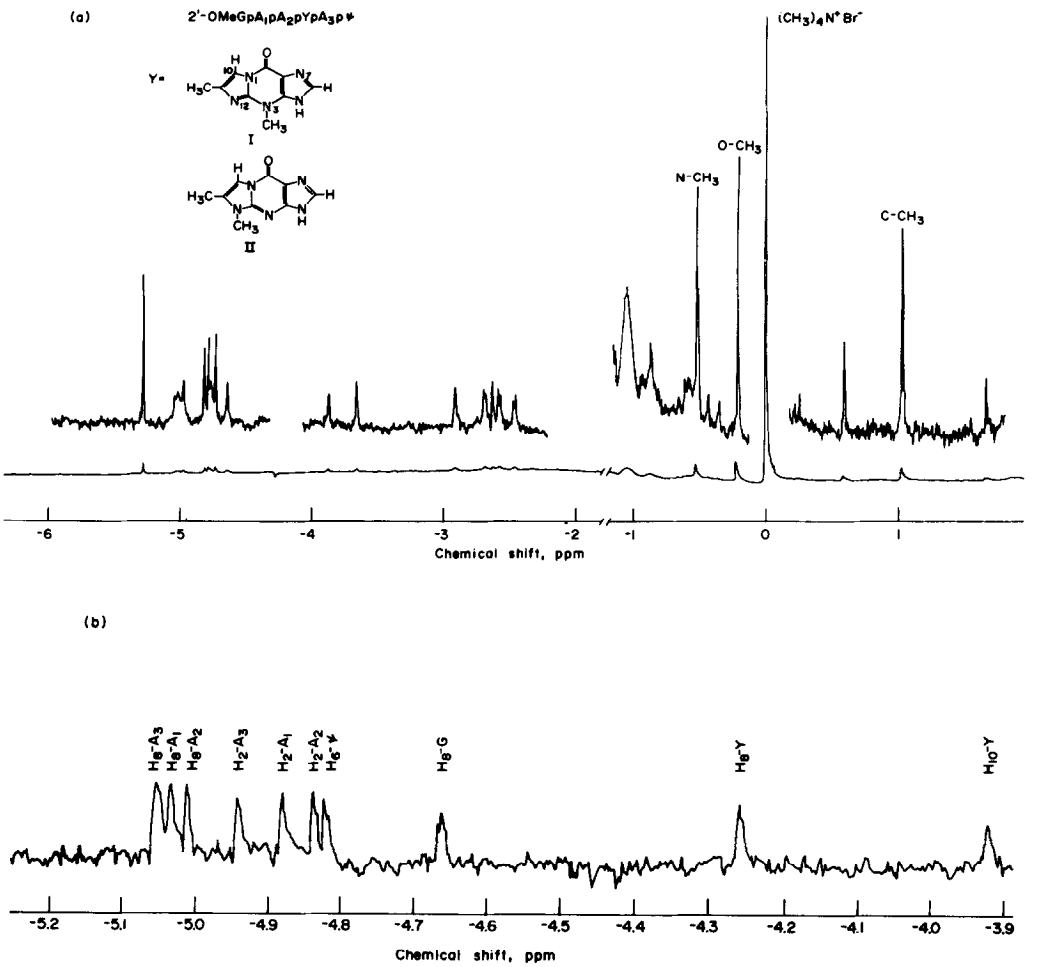

Figure 1. (A) Typical 300 MHz spectrum at 2500 Hz spectral width of hexamer 2'-OMeGpApApYpApψ. 100 accumulations in Fourier transform mode using VFT-100-SC with 16K memory, probe temperature 24°C; $50A_{260}$ units in 0.5 ml D_2O in 0.01 M phosphate buffer, pH 7.0. EDTA(Na_2) was added to chelate paramagnetic ions, resulting in a solution of 10^{-5} M. Internal $(CH_3)_4\overset{+}{N}\ \bar{Br}$ used as reference. (B) Expansion of the aromatic proton region centered at caδ-4.5 ppm. Sample conditions same as in (A); 100 scans in FT mode; probe temperature 70°C, at which temperature minimum peak overlap occurs. The peak assignments are discussed in the text. Kasai et al. (3) did not rigorously show that the C-CH_3 was on either C_{10} or C_{11}, but preferred C_{11}.

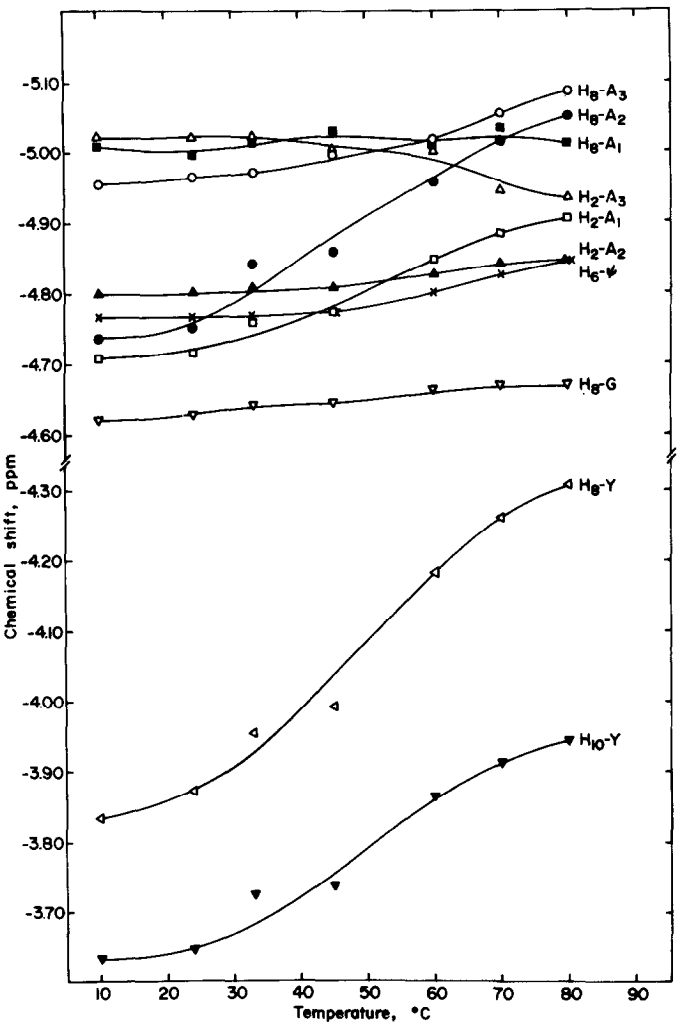

Figure 2. Chemical shift dependence upon temperature for aromatic protons of the hexanucleotide. Shifts measured from internal $(CH_3)_4\overset{+}{N}\ \bar{Br}$.

anti glycosyl conformation, and the bases stacking in a partial overlapping fashion.

Intrinsically, it is expected that the order of chemical shifts from low to high field should be: H_8-A < H_2-A < H_8-G(17) < H_8-Y < H_{10}-Y(3). This consideration with the temperature data leads to assignment of H_{10}-Y at highest field. This assignment is consistent with the broader line width exhibited by this proton due to the splitting caused by the adjacent methyl protons. At

next highest field, the proton assigned H_8-Y is particularly sensitive to temperature change. Over the range considered, the $\Delta\delta$ is 0.48 ppm, which is 40-50% greater than the temperature effects upon any base protons in trimers such as ApApA and ApApG (18). We rationalize the unusually large effect in terms of a change in population of conformers about the glycosyl bond in the Y nucleoside from mainly syn to anti in character. This continuum of change from syn to anti conformation with temperature rise takes the H_8-Y proton from a highly shielded environment sandwiched between the diamagnetic bases of A_2 and A_3 to a deshielding situation generated by the field of the internucleotide phosphate between nucleosides A_2 and Y. During this proposed syn⟶anti flip, the pyrrole proton at C_{10} is removed from the ring current generated by the neighboring base A_2, resulting in a similar downfield shift but of less magnitude (0.31 ppm).

Expected to show little temperature effect and occur at high field is H_8-G where there is no neighboring base and no deshielding phosphate. With respect to the curves at 80°C, H_8-G then is next highest field to H_8-Y. Proceeding downfield further at 80°C, the next curve which shows some effect due to base overlap from neighboring A_3, is tentatively assigned to H_6-ψ.

Examination of the molecular model proposed for the hexanucleotide at low temperature where the Y nucleoside is in the syn conformation serves to illustrate that H_2-A_3 is distant from any diamagnetic purine ring and is expected to experience a downfield shift from the 6-keto anisotropy of the Y-base. As temperature is raised and the population of Y anti conformer is increased, both H_2-A_3 and H_2-A_2 are expected to experience ring current shielding from the Y-base and thus should display shifts to higher field at high temperatures, leading to the assignment for H_2-A_3. This upfield shift is not apparent in H_2-A_2 since this proton also experiences a decrease in shielding as A_1 unstacks. H_2-A_1 is expected to be the H_2 at highest field at low temperatures because it is the only one under another adenine ring. The curve assigned to H_2-A_1 also shows a typical H_2 temperature dependence of 0.20 ppm (18) over the range studied.

The remaining protons to be assigned are the H_8's of the adenine bases, which would be expected to appear at low field. The relatively upfield chemical shift for H_8-A_2 at low temperatures is a good indication of the proximity of the diamagnetic ring of the Y-base, and this is only possible if the Y-base is in the syn conformation. Chemical shifts of H_8-A_2 and H_8-A_3 would be expected to display considerable temperature dependence due to overlap with neighboring purine bases. Since H_8-A_2 is flanked by bases of A_1 and Y, the temperature dependence of this proton should be greater than H_8-A_3, leading to their respective assignments. The methyl at the 2'-position of the 3'-terminal guanosine may prevent efficient overlap of guanine and the adenine ring of the adjacent A_1, therefore H_8-A_1 would be expected to exhibit minimal temperature dependence, leading to the assignment of this proton.

We have proposed the syn⟶anti conformational flip for the Y nucleoside in order to rationalize the observed chemical shift vs temperature data. In considering the proposed structure for the Torula Y-base (3; fig. 1, structure I) it is highly unlikely that the N-3 methyl group could be sterically accommodated over the ribose ring when the Y nucleoside assumes the syn glycosyl conformational preference at low solution temperature. We therefore propose that structure II, figure 1, in which the methyl is at N-12 rather than N-3 is more compatible, and would certainly sterically allow a syn⟶anti flip as proposed. A possible objection to this assignment may lie with the small differences in uv at acid pH found between N-3 and N-12 substituted guanines of this nature (2). However, the models synthesized by Furutachi, et al. were not also alkylated at N-9 as in the case of the Y nucleoside.

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