NMR spectroscopy of the ring nitrogen protons of uracil and substituted uracils; relevance to A Ψ base pairing in the solution structure of transfer RNA

R. E. Hurd and B.R. Reid

Biochemistry Department, University of California, Riverside, CA 92502, USA

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ABSTRACT

The chemical shifts of the well-resolved ring nitrogen protons of uracil and a series of substituted uracils, including pseudouridine (5-ribosyl-uracil) and uridine (1-ribosyl-uracil) were determined using nuclear magnetic resonance (NMR). The observed chemical shifts suggest the existence of an atypical syn conformation for pseudouridine in the A Ψ base pair in regulatory tRNAs in solution.

INTRODUCTION

The presence of an A Ψ base pair at the external terminus of the anticodon helix of certain tRNAs appears to be an essential structural requirement for the genetic control of the operon for the biosynthesis of the corresponding amino acid [1, 2].

Our analyses of the low field NMR spectra of ring NH hydrogen bonds of several tRNA species which contain such an A Ψ base pair consistently indicate an anomalously upfield starting position for the inherent chemical shift of the A Ψ ring NH resonance (a minimum of 0.60 ppm higher field than the inherent unshifted starting position of a standard AU resonance) (3). This anomalous difference between the A Ψ^* and the AU * unshifted starting positions is supported by our assignment of the A Ψ base pair resonances in the low-field NMR spectra of yeast tRNA^{Phe}, yeast tRNA^{Trp}, E. coli tRNA^{His}, E. coli tRNA^{Phe}, E. coli tRNA^{Lys} and E. coli tRNA^{Met}_m. Furthermore the simpler spectra of isolated tRNA hairpin helices which contain an A Ψ base pair also provide evidence for the A Ψ^* -AU * resonance discrepancy. For example we have studied the anticodon helix of yeast tRNA^{Phe} (4), and the yeast tRNA^{Leu}_{UUG} (5) anticodon helix has been studied by others; both helices were found to contain no resonance lower than -13.3 ppm. In both helices the upfield ring current shifts on the A Ψ pair by stacked neighbors are estimated to be ca. 0.4 ppm. In fact the application of fundamental ring current shift theory combined with the crystallographic coordinates of yeast tRNA^{Phe} lead to a value of less than 0.4 ppm upfield shift from all neighbors on the A Ψ base pair (6). Thus the

inherent unshifted position for $A\psi^\circ$ appears to be ca. -13.7 ppm; this is 0.65 ppm higher field than the AU° chemical shift.

For AU base pairs in tRNA the upfield ring current shifts on the U N3H proton from stacked neighbors can only be correlated with experimentally observed resonance positions when a value of -14.35 ppm is chosen for the unshifted inherent AU° position (the unshifted inherent GC° chemical shift is approximately -13.5 ppm). This AU° position is further corroborated in the simpler spectra of isolated hairpin helices containing AU resonances in a variety of nearest neighbor environments. An important contribution to the extra downfield shift on the N3H of uridine in a UA pair compared to the N1H of guanosine in a GC pair is the in-plane ring current of the complementary adenine; this in-plane shift should be the same in AU pairs and in $A\psi$ pairs. Hence we reasoned that the ca. 0.6 ppm difference between the unshifted inherent $A\psi^\circ$ and AU° positions might be evident in a comparison of NMR spectra of the respective free monomers i.e., uridine versus pseudouridine.

In this study, the high resolution (360 MHz) proton NMR spectrum of uracil in d_6 -DMSO, under conditions where both the N1H and the N3H ring nitrogen protons are well resolved, is compared in a coherent series with the following substituted uracils; thymine (5-Me-uracil), pseudouridine (5-ribosyl-uracil), uridine (1-ribosyl-uracil), and thymidine (1-ribosyl-5-Me-uracil). Arguments based on the chemical shifts of the ring NH protons presented in this investigation are completely consistent with our hypothesis that, in tRNA, the $A\psi$ base pair exists in the atypical syn conformation i.e. the N1H rather than the expected N3H of pseudouridine is hydrogen bonded to adenosine.

MATERIALS AND METHODS

Uracil, thymine and thymidine were purchased from P. L. Biochemicals. Uridine and the α and β anomers of pseudouridine were obtained from Sigma. 5 mm NMR Tubes (527-PP) and d_6 -DMSO were products of Wilmad Glass Company, Inc., Buena, N. J.

Samples were prepared in the following manner; 0.05 mmole and 0.005 mmole of each of the monomers were weighed out as dry powders. 2 ml of double distilled H_2O was added to each sample and the pH of the resulting solution or suspension adjusted to 3.0. These samples were then lyophilized and re-suspended in 0.5 ml of dry d_6 -DMSO.

Proton NMR spectra, were obtained on a Bruker HXS 360 spectrometer at the Stanford Magnetic Resonance Laboratory. The average free induction decay (FID), collected from 4 quadrature mode pulses of 13.5 microseconds duration each, and separated by 20 sec (over five times the longest T_1 observed), was

Fourier transformed, phased, and printed directly on white paper for the figures presented.

The "pH4" spectrum of uracil was collected under similar conditions, but was run at 90 MHz on a Bruker WH90 spectrometer in the Chemistry Department at the University of California, Riverside. Chemical shifts for the monomer spectra are reported as ppm from TMS.

RESULTS

The proton NMR spectrum of uracil in dry d_6 -DMSO, "pH3", has both ring NH protons resolved (Fig. 1). As a result of the deshielding effects of two adjacent carbonyls, N3H is expected to be the lower field peak of these two resonances. N1H should be at higher field since it is only deshielded by the C2 carbonyl. This assignment is in agreement with previous NMR studies [7, 8]. Interestingly, scale expansion of the 7-8 ppm region revealed that the C6H is a quartet under these conditions whereas under conditions where N1H and N3H are exchanging too rapidly to be resolved (higher pH and less dry solvent) C6H is a doublet with a 7.5 Hz coupling constant (Fig. 2). These data suggest that C6H is coupled to both C5H and N1H when exchange of N1H is sufficiently slow. Moreover, the two coupling constants which cause the C6H quartet are $J(C5H-C6H) = 7.5\text{Hz}$ and $J(N1H-C6H) = 6\text{Hz}$. The broader linewidth of the higher field N1H resonance also reflects the N1H-C6H coupling (Figs. 1 and 3).

A methyl replacement for C5H (thymine) produces no change in the chemical shift of the lower field N3H ring nitrogen proton (Fig. 3a, 3b). N1H, the higher field of the two ring nitrogen protons, and C6H undergo upfield shifts;

Figure 1. The 360 MHz proton NMR spectrum of uracil (0.1 M, "pH 3") in dry d_6 -DMSO. The spectral region between -7 ppm and -12 ppm contains the aromatic C6H proton and the water-exchangeable N1H and N3H protons. Expansion of the -7 to -8 ppm region reveals a C6H quartet with peaks at -7.391, -7.408, -7.411 and -7.428 ppm.

Figure 2. The lowfield (-7 to -12 ppm) 90 MHz proton NMR spectrum of uracil (0.1 M, "pH 4") in d_6 -DMSO. Less rigorous precautions to ensure the dryness of the sample and solvent were taken compared to the spectrum in Figure 1.

Figure 3. The lowfield (-7 to -12 ppm) 360 MHz proton NMR spectra of several substituted uracils (0.1 M, "pH 3") in dry d_6 -DMSO. a) uracil, b) 5-methyl-uracil, c) 5-ribosyl-uracil, d) 1-ribosyl-uracil, e) 5-methyl-1-ribosyl-uracil. The C6H proton is a quartet in a), a doublet in d) and a singlet in the remaining spectra.

C6H moves up by 0.16 ppm and N1H moves up by 0.18 ppm. The C6H quartet of uracil collapses to a singlet in thymine, an effect which can be interpreted as the loss of the C5H-C6H coupling due to the substitution of the C5H proton with a methyl group plus the loss of the N1H-C6H coupling by N1H exchange. Comparison of the uridine and thymidine spectra also demonstrates the effect of replacing C5H with a methyl group. Consistent with the thymine-uracil data, N3H is only slightly shifted, whereas a 0.19 ppm upfield shift is suffered by the C6H resonance (Fig. 3d, 3e) and the coupling with C5H is of course abolished.

Replacing the N1H proton of uracil with a ribose (uridine) results in a downfield shift of both N3H (by 0.37 ppm) and C6H (by 0.51 ppm) as shown in Fig. 3a, 3d. An analogous effect is seen for thymidine versus thymine; N3H shifts downfield by 0.32 ppm and C6H is downfield shifted by 0.48 ppm (Fig. 3b, 3e). A C5H coupling of 8Hz is observed for the C6H doublet of uridine.

The spectrum of pseudouridine (5-ribosyl -uracil) shows the effect of a ribose substitution at the C5 position of uracil. N1H-C6H coupling is evident from the broadened linewidth of the C6H resonance in Fig. 3c. In support of this observation, a pseudouridine sample containing a higher H_2O to nucleoside ratio, and hence greater N1H-solvent exchange, was found to have a much narrower C6H linewidth (Fig. 4). The unnatural nucleoside, α -pseudouridine

Figure 4. The lowfield (-7 to -12 ppm) 360 MHz proton NMR spectrum of β -pseudouridine (0.01 M, "pH 3") in d_6 -DMSO; this sample was slightly less anhydrous than the sample in Figure 3c.

Figure 5. The lowfield (-7 to -12 ppm) 360 MHz proton NMR spectrum of α -pseudouridine (0.1 M, "pH 3") in dry d_6 -DMSO.

(all other nucleosides discussed in this paper are the naturally occurring β anomers), presents a better example of the N1H-C6H coupling. One observes a C6H doublet and a N1H doublet with identical coupling constants (N1H-C6H) of 6Hz in the NMR spectrum of α pseudouridine (Fig. 5).

The net effect on the C6H resonance by ribose substitution at the C5 position of uracil (β pseudouridine) is a 0.15 ppm downfield shift. As suggested by Hruska et. al. [9], this may reflect the opposing effects of a through-space electric field effect from the hemiacetal oxygen [10], and a through-bond substituent effect. It is interesting to note that the C6H of α pseudouridine appears to lose the downfield effect, since this C6H resonance is observed 0.25 ppm upfield of the corresponding resonance in uracil.

An important observation is that the chemical shift of the N3H proton of β pseudouridine (the proton normally expected to hydrogen bond to adenosine in the typical anti conformation) is only 0.20 ppm higher field than the N3H proton of uridine and this is inadequate to explain the anomalously upfield shifted (0.6 ppm) $A\psi^\circ$ resonance position. The β pseudouridine N1H proton is more than 0.40 ppm upfield compared to the N3H of uridine. Hence the upfield position observed for the $A\psi$ ring NH resonance in tRNA cannot be explained by N3H hydrogen bonding of pseudouridine to adenosine; it can, however, be explained by hydrogen bonding the pseudouridine N1H to adenosine. The only way in which pseudouridine N1H can be brought into position to hydrogen bond with adenosine is to rotate the glycosidic bond by 180° from the normal anti conformation to the atypical syn conformation as shown in Figure 6. Based on this data we propose that adenosine is hydrogen bonded to the higher field ring

ψ (anti) Watson-Crick pairing with A.

ψ (syn) atypical pairing with A.

Figure 6. Normal (anti) and atypical (syn) base pairing of pseudouridine with adenosine. The syn conformation is generated by a 180° rotation of the pseudouridine glycosidic bond.

NH proton of pseudouridine (N1H) which must exist in the atypical syn conformation in the $A\psi$ base pair found at the end of the anticodon helix in regulatory tRNAs.

DISCUSSION

Even the difference between the chemical shifts of N1H of pseudouridine and the N3H of uridine (0.40 ppm) is not quite enough to completely account for the different resonance positions of $A\psi^\circ$ and AU° base pairs (0.6 ppm). This may reflect the fact that monomer spectra represent predominantly anti conformations [9], which is the correct orientation for the AU pair, but incorrect for the $A\psi$ pair in tRNA. Rotation of the N1H from its position above the ribose, as it is found in the anti conformation of pseudouridine, to the syn position away from the ribose, would result in an upfield shift (due to the loss of the deshielding electric field effect produced by the ribose oxygen [10]). With this extra correction, the $A\psi^\circ$ versus AU° difference is very nicely accounted for by the proposed syn conformation of $A\psi$ base pairs.

This unique ability of pseudouridine to use its N1 proton for hydrogen

bonding to adenosine may be the structural basis for the regulatory function of the A Ψ pair. This atypical base pairing generates a new ring NH donor (N3H) and an adjacent carbonyl oxygen acceptor in the major groove of the anticodon helix, a consequence which might stabilize a conformation which is necessary for the regulatory function of these tRNA species.

We suggested the syn pseudouridine conformation in the A31. Ψ 39 base pair to Quigley and Rich who have examined the tRNA^{Phe} crystal structure in this light (11). They report that further refinement of the electron density is required to unambiguously answer this question in the crystal structure. However an interesting point is that, in agreement with our prediction, the electron density of the other carbonyl oxygen of pseudouridine (which they call O2 instead of O4) appears to be in the groove pointing away from adenine rather than pointing towards adenine in the more normal type of pairing (11).

An important consideration to be made is that a subtle change in only one nucleotide involving transfer of the ribose glycosidic linkage from the N1 to the C5 position enables certain tRNA molecules to participate in an important biological function, namely the genetic control of the enzymes of amino acid biosynthesis. It would appear that U39 should be capable of any structural functions carried out by Ψ 39 if the latter is in the normal anti conformation. Thus it seems likely that Ψ 39 in a syn conformation would be necessary to provide the structural difference required for the "extra" biological function unique to regulatory tRNAs.

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