NMR INVESTIGATION OF PROTON EXCHANGE IN TRANSFER RNA BY HIGH RESOLUTION NMR.

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SUMMARY: High resolution (300 MHz) nmr has been used to follow the exchange-out of ring NH protons involved in Watson-Crick secondary structure base pairs in yeast $tRNA_3^{Leu}$ and $tRNA^{Phe}$. At 4°C the ~20 resonances observed in the low field region of the spectrum (11 to 15 ppm) exchange with the solvent (D_2O) in less than 300 sec. Because the exchange-out is so rapid, we are unable to follow the behavior of imino protons in specific Watson-Crick base pairs. The 20-40 very slowly (1 hr.) exchanging protons observed by tritium exchange must be assigned to amino protons.

INTRODUCTION

Hydrogen exchange has played an important role in studies of protein and polynucleotide conformation because rates of exchange are very sensitive to conformation (1). With double helical polynucleotides exchange rates of amino and imino protons with water are slow enough to be studied by tritium exchange gel filtration methods (2-5). In the case of tRNAs, about 110 slowly exchanging protons can be detected, with exchange times ranging from seconds to several thousand seconds at 0°C in solutions containing high salt and magnesium (6-8). Of this total, a group of about 20 very slowly exchanging (1-2 hrs.) are especially interesting since this number is close to the number of secondary structure base pairs (8). If the different classes of slowly exchanging

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protons could be assigned to specific types of hydrogen bonded protons (e.g., ring NH or amino protons in secondary or tertiary structure base pairs) then tritium exchange experiments would be even more valuable as a tool for studying RNA structure. Unfortunately, this has not previously been possible. However, since the exchange rates are relatively slow it is possible to use high resolution nmr to directly observe individual hydrogen bonded ring NH and amino protons (9,10).

In the present study high resolution nmr was used to investigate the selective exchange of ring NH protons from individual Watson-Crick base pairs in two different tRNA molecules. In these experiments the time dependence of resonances in the low field spectrum were measured after rapidly dissolving a sample of tRNA in D_2O. We were particularly interested in the possibility that the ~20 slowest exchanging in protons might be due to the ring NH protons in Watson-Crick base pairs, and that the observation of slow, sequential exchange with D_2O solvent protons might eventually help in the assignment of the low field nmr spectra of the tRNA and provide information about the dynamics of the hydrogen exchange process itself.

MATERIALS AND METHODS

Purified yeast $tRNA_3^{Leu}$ was kindly supplied to us by Dr. Simon H. Chang, Louisiana State University. Based on [^{14}C] leucine acceptance and T_1 ribonuclease degradation, it was judged to be greater than 90% pure. Yeast $tRNA^{Phe}$ was kindly prepared by Mr. P. Bolton in our laboratory (98% pure). Transfer RNA samples were extensively dialyzed against 10 mM NaCl and double distilled water, before the addition of, or dialysis against the final buffer to prevent contamination by hydrogen exchange catalysts (11).

Nmr spectra were obtained with a Varian HR 300 spectrometer operating in field sweep mode and a Nicolet 1020A signal averager was used to accumulate spectra. Near saturating power levels (40 dB) and short sweep times (10 sec/1000 Hz) were used to rapidly (100 sec) accumulate spectra (Figure 1 insert).

Samples of tRNA (~4 mg) in 125 ± 10 μl buffer containing 0.1 M (or 3 M) NaCl, 10 mM sodium cacodylic acid (pH 7.0) and 10

mM $MgCl_2$ in H_2O were concentrated in the nmr tube by a stream of nitrogen to a final volume of 25 ± 10 µl, after running a low field nmr spectrum in H_2O to determine the initial spectral intensity of the sample. 100 µl of precooled D_2O (4°C) was added to the frozen sample (D_2O buffer at -5°C with high salt experiments), and the solution was thoroughly mixed by vortexing the nmr tube at 0°C (-5°C) (centrifuge tube with drilled stopper containing ice water and acetone served as a cooling jacket). The nmr tube was then inserted into the spectrometer probe maintained at 4 ± 1.5°C (0 ± 1°C). The spectrometer was pretuned on a sample with similar tuning characteristics, so only a minor retuning was necessary before collecting spectra. Data collection started 3 to 5 min. after the D_2O addition.

RESULTS AND DISCUSSION

The time dependence of the low field nmr spectrum (4 ± 1.5°C) of a sample of yeast $tRNA_3^{Leu}$ after five fold dilution with D_2O is shown in Figure 1b-f. Although the quality of the initial spectra is very poor due to the short signal averaging times (2 minutes, 10 sweeps), it is clear that exchange of the hydrogen bonded ring NH protons is nearly complete after only 4 ± 1 minute (compare intensity in 1a with 1b-f). No significant change in intensity occurs after exchange times of up to 45 minutes, and the remaining intensity was shown to be exclusively due to the residual water content of the sample (20%) by running an additional spectrum after reannealing the sample at room temperature.

We also carried out several D_2O exchange experiments with yeast $tRNA_3^{Leu}$ and yeast $tRNA^{Phe}$ in solutions containing 10v% EtOH or 3 M NaCl (and 10 mM $MgCl_2$ dialyzed in) and at temperatures as low as -5°C in an attempt to further reduce the exchange rates of the hydrogen bonded ring NH protons). The results of an exchange experiment with $tRNA^{Phe}$ in 3 M NaCl at 0 ± 1.5°C are shown in Figure 2, and again it is evident that complete exchange-out has occurred within ~300 seconds.

We attempted to observe slow exchange of amino protons in the spectral region between 6 and 7.5 ppm, but failed because of close proximity to the residual water peak and to the aromatic resonances

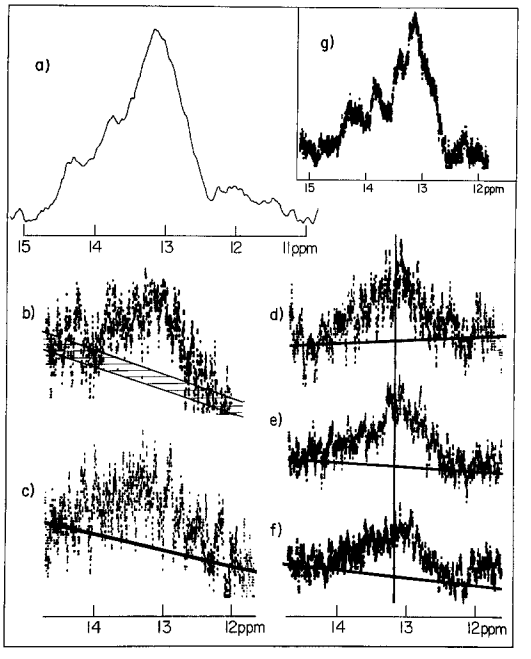

Figure 1. D_2O exchange of yeast $tRNA_3^{Leu}$ at 4 ± 1.5°C (0.1 M NaCl, 10 mM cacodylate, 10 mM $MgCl_2$, pH 7.0). The spectrum in (a) was obtained by extensive signal averaging at 4°C and then displayed on a chart recorder. The spectra shown in (b) and (f) were recorded by photographing the oscillograph output of the signal averager, and are shown on the same gain and sweepwidth as the spectrum shown in (a) so that the intensities in spectra (a) to (f) can be directly compared. The times at which these spectra were recorded following sample preparation were as follows: (b) 180 to 310 sec., 10 sweeps; (c) 330 to 435 sec., 10 sweeps; (d) 450 to 695 sec., 20 sweeps; (e) 1000 to 1505 sec., 40 sweeps; (f) 2000 to 2575 sec. after D_2O addition, 50 sweeps. Estimated baselines have been included in b-f. To illustrate the quality of the spectra which can be obtained in H_2O by rapid averaging, a spectrum of yeast $tRNA_3^{Leu}$ obtained at 23°C using only 10 sweeps of 10 sec. each is shown in insert (g).

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of tRNA, which prevented the use of high RF power. The strong curvature of the "baseline" even under reduced power levels (30 dB) rendered the detection of a small number of resonances (20) very difficult even after extensive signal averaging.

In comparing our nmr results with previous studies it should

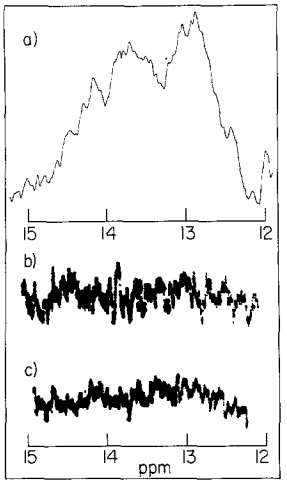

Figure 2. D_2O exchange of yeast $tRNA^{Phe}$ at 0 ± 1.5°C (3 M NaCl, 10 mM cacodylate, 10 mM $MgCl_2$, pH 7.0): low field nmr spectrum (a) in H_2O; (b) 270 to 570 sec., 50 sweeps, (c) 1205 to 3580 sec. after D_2O addition, 400 sweeps. All three spectra are recorded using the same gain and sweepwidth so that they may be directly compared.

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be noted that Englander and Englanders' tritium exchange results were independent of tRNA concentration over a range from 1 to 16 mg/ml (7). Furthermore, McConnell and von Hippel observed no catalytic effect by negatively charged proton transfer agents, such as cacodylate or phosphate, on the hydrogen exchange of DNA at neutral pH (11,12). We conclude, therefore, that our nmr exchange experiments (Figures 1,2) should be directly comparable with the tritium exchange experiments which were carried out at somewhat lower tRNA concentration.

Webb and Fresco (8) previously used the tritium exchange method to study slowly exchanging protons in yeast $tRNA_3^{Leu}$ under conditions very similar to those used in our study. They find approximately 110 slowly exchanging protons which can be divided into two groups: approximately 70 have a half life of about 150 seconds and a second group of 40 has a half life of about 20 min-

utes. Our results permit us to assign ~20 of the more rapidly (less than 300 seconds) exchanging protons to the ring NH protons in secondary structure Watson-Crick base pairs. The group of 40 protons with half life of 20 minutes must therefore be assigned to slowly exchanging amino protons. Unfortunately, we were not able to follow the exchange-out of individual resonances in the low field spectrum, so we are unable at this time to assign half lives to protons in specific base pairs. This probably can be accomplished using other nmr techniques such as spin saturation (13,14) and such experiments are in progress.

Acknowledgment

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