^{31}P MAGNETIC RESONANCE OF PURIFIED tRNA

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1. Introduction

We report the first observation of well-structured ^{31}P magnetic resonance spectra from two purified tRNAs. Although generally similar, the spectra are distinct. They are affected by pH and by melting. Preliminary evidence is in agreement with specific binding of Mg^{2+}. ^{31}P resonance could be an excellent tool for structural and functional studies of tRNA.

2. Materials and methods

E. coli $tRNA^{glu}$ was from Oak Ridge National Laboratory, batch No. 15291. Twenty mg in the original 1 M NaCl solution were dialysed against 4 l of double-distilled water with a suspension of Chelex, for 8 hr, repeated once. After lyophilization, the tRNA was dissolved in 1 ml D_2O (concentration of tRNA ~ 8×10^{-4} M) with 5×10^{-4} M cacodylate buffer and 5×10^{-3} M tetramethylammonium chloride (TMA). The solvent had been previously washed with Chelex in order to eliminate divalent ions. The glassware (NMR tubes) was cleaned with sulfochromic acid.

Yeast $tRNA^{phe}$ was from Boehringer. It was treated as above, except that the last dialysis was against 10^{-4} M EDTA. Fifteen mg were dissolved in 0.8 ml.

The NMR spectra were taken with a Varian XL-100 (phosphorus frequency 40 MHz). We used a deuterium internal lock and proton noise decoupling. A teflon plug (Wilmad) was inserted in the 12 mm sample tubes to avoid vortices. A Nicolet computer was used for data accumulation.

3. Results

Fig. 1 shows spectra obtained with unfractionated tRNA (40 mg in 1 ml). There are some poorly resolved features, notably a tail on the low field side (left) and a shoulder to the right. The linewidth is ~26 Hz. The non-decoupled spectrum shows the influence of the coupling between ^{31}P and the 3′ and 5′ sugar protons: it is broadened by ~5 Hz. This is a reasonable value. For comparison we observed a 7.4 Hz splitting in the

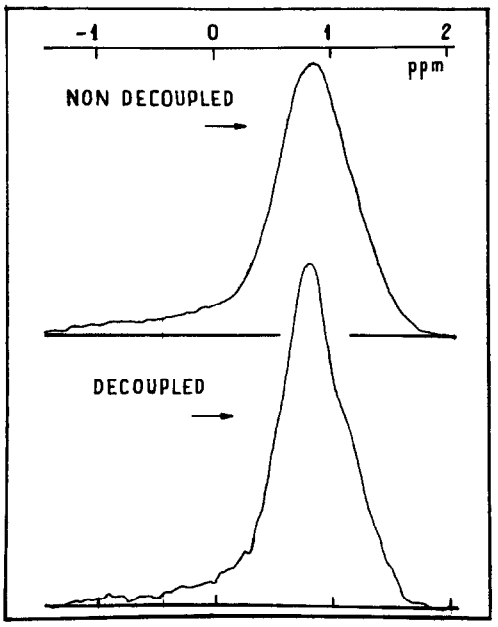

Fig. 1. ^{31}P spectrum of unfractionated tRNA. The external reference was $P(OCH_3)_3$ whose chemical shift relative to PO_4H_3 is −140.0 ppm [2]; PO_4H_3 is taken as origin of the chemical shifts (positive towards high fields).

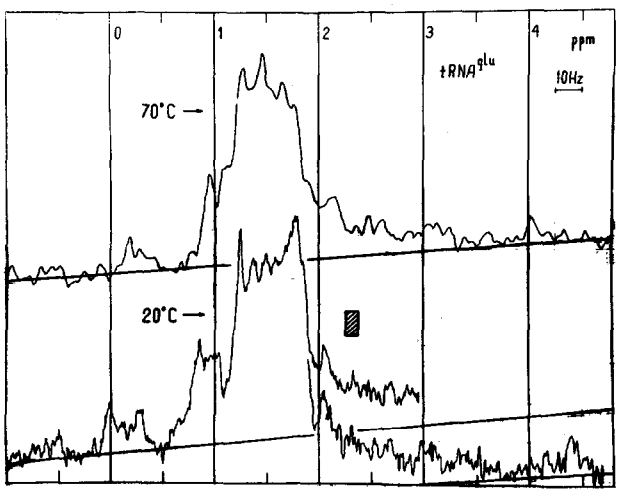

Fig. 2. ^{31}P spectrum of *E. coli* $tRNA^{glu}$. The area of the hatched rectangle corresponds to the resonance of one phosphate, as computed from the total area of the spectrum which corresponds to ~80 phosphates. Certain lines (e.g. that at 2.05 ppm) correspond to a single phosphate. The resolution of the main 20° spectrum is below the intrinsic one due to the use of a slightly saturating rf field. The trace from 1.9 to 4.7 ppm was obtained on the same sample also at 20°. Conditions for the 20° spectrum: pD = 6.4, accumulation time: 4 hr, rf power = 90 db. For the 70° spectrum: 13 hr, rf power = 85 db.

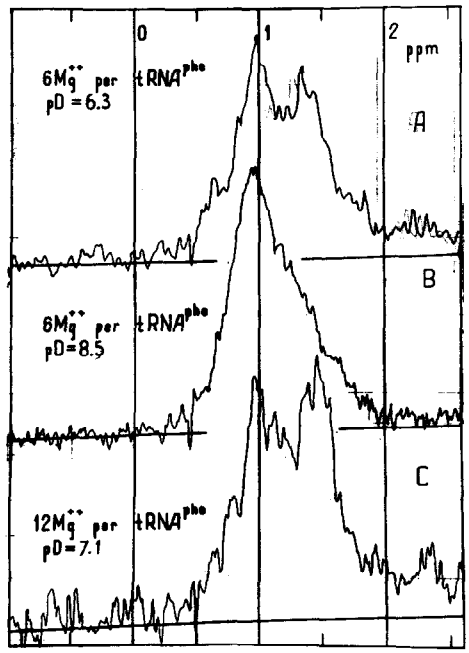

Fig. 3. ^{31}P spectrum of yeast $tRNA^{phe}$. It is similar to that of $tRNA^{glu}$ but not identical (see text). There is a drastic pH effect. The time sequence is A, B, C.

^{31}P resonance of 3′-AMP. In 3′-5′ ApA the H-^{31}P couplings are 3.4, 6.5 and 8.1 Hz [1].

In fig. 2 is shown the highly resolved spectrum of *E. coli* $tRNA^{glu}$. Most of the intensity (55%) is concentrated in one main region between 1.1 and 2 ppm. Note the sharp limits (vertical "walls") of this region. Two clusters of lines are centered at 0.1 and 0.9 ppm. Beyond 2 ppm there is appreciable intensity in a broad tail extending to 3.5 ppm, and narrow lines appear at 2.05, 2.7 and 4.4 ppm. At least 13 distinct lines are observed, with linewidths for some of them as small as 2 Hz.

Upon heating to 70°, which should result in complete melting in our conditions, the general features of the spectrum are conserved. However, there are some definite changes. The shape of the main region changes, the peak at 4.7 ppm is shifted and the clusters at 0.1 and 0.9 ppm seem to lose intensity. All lines broaden.

The spectrum of yeast $tRNA^{phe}$ (fig. 3) is similar to that of *E. coli* $tRNA^{glu}$ but there are clear differences, both in and out of the main region. The main region is at slightly lower field than for $tRNA^{glu}$, and it accounts for a larger part of the total intensity. Upon raising the pD (measured pH + 0.4) by addition of NaOH, drastic changes occur. Much resolution is lost and the intensity distribution in the main region changes.

4. Discussion

All the phosphates of tRNA are linked in identical 3′-5′ diester bonds, and one might have guessed that they should therefore all have the same chemical shift, thus giving a spectrum consisting of only one line. Various effects may contribute to selective chemical shifts of the different phosphates: (1) Base specific shifts. The different mononucleotides differ in their chemical shifts by up to 1 ppm [3]. The diamagnetic shift may contribute to these differences. The diamagnetic contribution should be larger in the mononucleo-

tides, in which the phosphate may come near the base, than in most bases of tRNA, notably those in a double helical part of the polymer, since the bases are then held distant from their linking phosphate. (2) The geometry of the phosphodiester linkage varies depending on whether one is in a double helical region, in a loop, or possibly in very distorted sites of the chain. The ^{31}P chemical shifts could be sensitive to such variations.

Our cautious feeling at this time is that the main region corresponds to phosphates participating in reasonably undistorted phosphodiester bonds, that is, phosphates in double helical regions and in unkinked parts of the loops. The other resonances would be assigned to phosphates in peculiar and constrained locations, which arise in the folding of tRNA in its native structure.

This picture is supported by the behavior observed upon melting (fig. 2). The main region is basically unchanged, showing that the corresponding chemical shift is largely independent of the ordered structure, whereas intensity is lost outside the main region, indicating that those chemical shifts are related to the ordered structure.

The large effects of changing pD from 6.3 to 8.5 (fig. 3: A, B) surprised us since neither the 3′-5′ linked phosphates nor the unmodified bases titrate in this range. This effect plagued our efforts to observe selective binding of Mg^{2+} to tRNA. Nevertheless, we observed that the addition of 6 Mg^{2+} per $tRNA^{glu}$ made a distinct change in the 0.9 ppm cluster without affecting the rest of the spectrum. This is preliminary evidence for specific Mg^{2+} binding, in agreement with previous observations [4].

In conclusion, ^{31}P magnetic resonance appears well adapted to the study of tRNA conformation, notably because the critical geometrical features may turn out to be reflected preeminently in the spectrum.

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Note added in proof:

Large pH effects were also observed in previous experiments on the NMR of exchangeable protons in $tRNA^{phe}$ (D. Kearns and R.G. Schulman, private communication).

References

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