^{31}P–NMR STUDIES OF tRNA

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1. Introduction

Unlike other spectroscopic methods, ^{31}P-NMR provides direct information on the state of the phosphate residues of nucleic acids.

Gueron [1] measured the ^{31}P-NMR spectra of two individual tRNAs by routine techniques; the time of accumulation was 4 to 8 hr. The pulse excitation method combined with Fourier transformation makes it possible to obtain rapidly NMR spectra of tRNA characterized by good signal-to-noise ratio. Thus, the techniques enable one to study the processes of tRNA transformation in the course of chemical or enzymatic reactions, as well as to study the effects of higher structure in solutions of low concentrations.

The present communication is concerned with the possibilities of the pulse-excitation ^{31}P-NMR spectroscopy as a means of investigation of tRNA higher structure, chemical modification and enzymatic hydrolysis.

2. Experimental

Unfractionated yeast tRNA obtained in technological laboratory of the Novosibirsk Institute of Organic Chemistry was purified from polyphosphate admixture according to [2]. To remove the divalent cations, it was absorbed onto a DEAE-cellulose column, the column washed with 5×10^{-3} M EDTA and the tRNA eluted with 1 M NaCl–5×10^{-3} M EDTA and precipitated with redistilled ethanol.

Yeast valine tRNA was kindly given by Dr M. Rivkin (Novosibirsk, Institute of Organic Chemistry). Pancreatic ribonuclease (Leningrad Meat-Packing Plant) was purified according to [3]. Polyuridylic acid sodium salt (poly U) was kindly given by Dr V. Rite (Novosibirsk State University). Poly A from Reanal was used.

To identify the signals, authentic samples of uridine-(2′, 3′)-cyclophosphate sodium salt (U > p), of disodium adenosine-5′-monophosphate (AMP) and of the dinucleotide GpCp obtained in the Institute of Organic Chemistry, Novosibirsk were used.

The acetylation of tRNA was performed according to [4]. The ^{31}P-NMR spectra were recorded using the 'Bruker-Physik' HX-90/8–15 spectrometer with Fourier transformation; the frequency was 36.4 MHz; decoupling from proton resonance was employed throughout. The B-NC 12 computer was employed for the accumulation of the interferograms and for the Fourier transformation. D_2O signal was used for internal stabilization. The chemical shifts were calculated relative to the external standard –85% phosphoric acid.

3. Results and discussion

It is seen in fig. 1a that the spectrum of unfractionated yeast tRNA consists of two lines at 0.8 and –3.1 p.p.m. Obviously, the 0.8 p.p.m. signal belongs to the phosphodiester groupings. The chemical shift of the second signal is close to that of AMP; its intensity is 60–80 times smaller than that of the first signal. It

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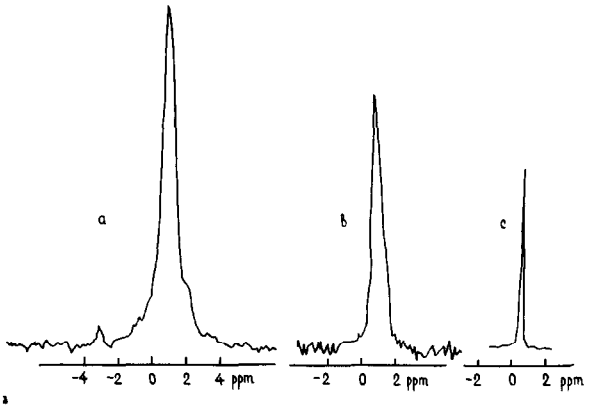

Fig. 1. a) Unfractionated tRNA (5×10^{-2} M) in buffer (Tris–HCl 0.1 M) at pH = 7.6; b) valine tRNA (2×10^{-2} M) under the same conditions; c) poly U (10^{-2} M) under the same conditions.

was attributed on this basis to the 5′-terminal phosphomonoester grouping. It is noteworthy that some tRNA preparations not subjected to purification from polyphosphate admixture also exhibited a group of signals at about 4.9 p.p.m.

Fig. 1b shows the ^{31}P-NMR spectrum of pure valine tRNA (yeast), a small amount of the substance was available. The signal of $tRNA^{val}$ phosphodiester groupings is similar to that of unfractionated tRNA, but the linewidth of the former is somewhat smaller than that of the latter.

It is seen in fig. 1a that the phosphodiester grouping signal of unfractionated tRNA has a linewidth of about 37 Hz. According to our calculations, the contribution of the dipole-dipole interactions of ^{31}P-nuclei to the linewidth did not exceed 0.05 Hz (the calculation was based on the mean distance between ^{31}P-nuclei 3.4 Å and on the known [5] rotational correlation time for the whole tRNA molecule). Due to the use of proton decoupling, it was possible not to consider the dipole–dipole interactions of ^{31}P-nuclei with protons. The fact that the contribution of the dipole-dipole interactions between ^{31}P-nuclei to the linewidth was small was confirmed by the small linewidth of the phosphodiester grouping signal of highly polymeric poly U (fig. 1c). It followed that the large linewidth of the tRNA resonance was only due to superposition of signals of slightly different chemical shifts.

Dispersion of chemical shifts could arise for the two following reasons: (i) because each phosphorus atom is in the neighbourhood of the primary structure of two chemically different nucleoside residues; (ii) because each phosphorus atom occurs in a particular orientation relative to some of the base residues distributed in an irregular manner in the complicated tertiary structure. Comparison of the spectra of poly A and poly U suggests that the difference of the chemical shifts due to the different chemical nature of the nucleotide residues is smaller than ca. 10 Hz.

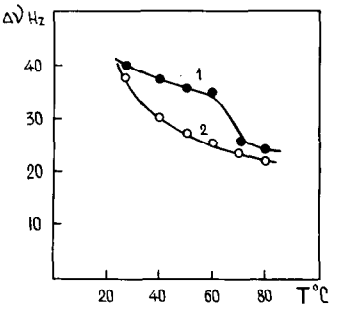

Fig. 2. tRNA Melting (1)-Tris–HCl 0.1 M, $MgSO_4$ – 0.15 M, NaCl–0.3 M, pH = 7.5; (2)-Tris–HCl 0.1 M, pH = 7.5.

On the other hand, it is seen in fig. 2 that thermal denaturation of tRNA does lead to a twofold decrease of the linewidth, the chemical shift being constant. It follows that a significant contribution to the linewidth of the ^{31}P-resonance of unfractionated tRNA is made by the different orientations of the phosphate residues relative to the base planes.

Fig. 2 shows the melting curves of tRNA under different conditions. It is seen that the major part of the decrease of the linewidth takes place at higher temperatures in the presence of Mg^{2+} ions; this is in accord with the fact that Mg^{2+} ions stabilize the tertiary structure of tRNA.*

Fig. 3 shows the spectra of tRNA acetylated to extents of 4% and 90% at the 2′-hydroxy groups of the ribose moieties. Acetylation leads to decrease of the ^{31}P resonance linewidth down to instrumental value of 5 Hz. Acetylation is known to result in

* Some tRNA preparations which were not subjected to purification from admixture of divalent cations exhibited an increase of the ^{31}P-resonance linewidth with increasing temperature; this may be due to the slow exchange of the ions between the binding sites on tRNA and aquocomplexes (cf. [7]).

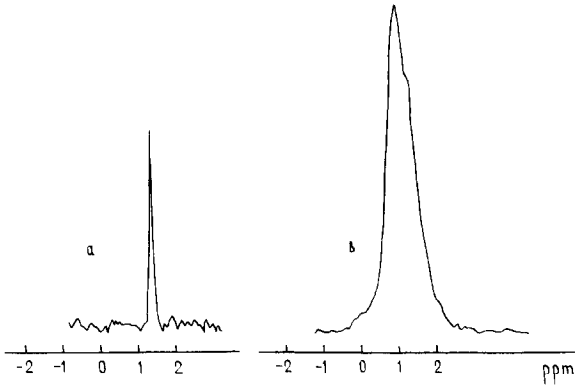

Fig. 3. ^{31}P-NMR spectra of the tRNA acetylation by 90% (a) and 4% (b).

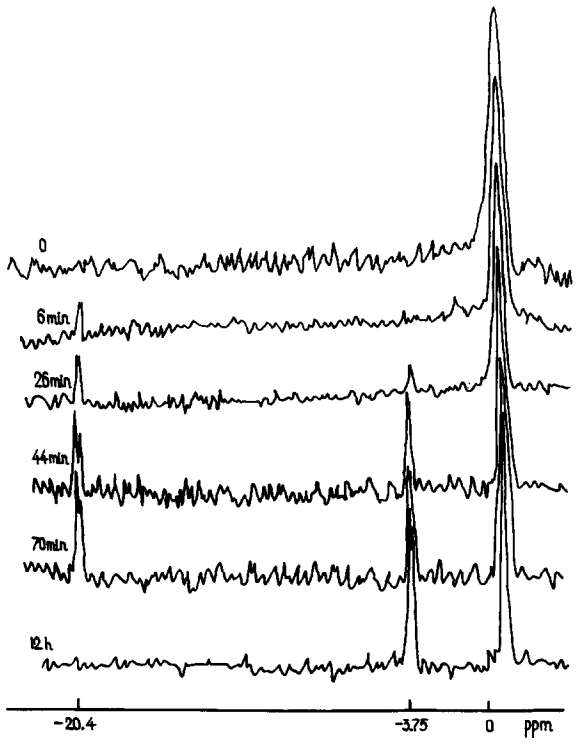

Fig. 4. tRNA hydrolysis with pancreatic ribonuclease $\frac{\text{tRNA concentration}}{\text{ribonuclease concentration}} = 8 \times 10^3$ (Tris–M HCl 0.1 M, pH = 7.7).

denaturation of tRNA [6]. As for the upfield shift of the ^{31}P-signal caused by acetylation, it can be caused by the influence of the electric field of the acetyl residue on phosphorus nuclei.

Fig. 4 shows the ^{31}P-NMR spectra taken in the course of the enzymatic hydrolysis of tRNA with pancreatic ribonuclease. The signal in the higher field is that of tRNA phosphodiester groupings. The chemical shift of the signal at −3.7 p.p.m. is close to that of the phosphate residue of nucleoside-3′-phosphates and of the 3′-terminal phosphate residue of the dinucleotide GpCp. The chemical shift of the signal in the lower field is the same as that of the signal of the phosphate residue of U > p. The pulse excitation NMR techniques thus made it possible to observe, in the course of enzymatic reactions, the signals of starting tRNA, of the intermediate nucleoside- and oligonucleotide-2′, 3′-cyclophosphates and of the final products nucleoside-3′-phosphates and oligonucleotides. NMR spectra revealed the intermediate products nucleoside-2′, 3′-cyclophosphates, hydrolysis of which to 3′-nucleotides is known to be [8] the limiting stage of the hydrolysis. The final extent of the transformation of phosphodiester into phosphomonoester groups which was determined in 12 hr of hydrolysis from the signal intensities was equal to 40%.

Acknowledgements

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