CODON–ANTICODON INTERACTION IN YEAST tRNA^{Phe}

An ^{1}H NMR study

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1. Introduction

The three dimensional structure of yeast tRNA^{Phe}, derived by X-ray diffraction studies [1,2], forms a good starting point for the investigations of structure–function relationships in tRNA. One of the outstanding features of this structure is that the DHU loop and the TΨC loop are connected by base pairs. As a result the TΨCG sequence is not available for pairing to other nucleotides. In solution this feature has been confirmed by oligonucleotide binding studies [3]. It was found that CGAA, which is complementary to the TΨCG sequence, does not bind to yeast tRNA^{Phe}. In addition nuclear magnetic resonance studies do indicate that the hydrogen bonds inferred from the X-ray structure are present in solution under the appropriate salt conditions [4].

Recent investigations into the mechanism of protein synthesis have suggested that the TΨCG sequence becomes linked to the ribosomal 5 S RNA during the ribosome binding process, meaning that the TΨC–DHU loop interaction becomes disrupted [5]. Moreover, it was found that significant binding of the oligonucleotide CGAA occurs when tRNA^{Phe} is complexed to $(pU)_8$ suggesting that the opening of the base pairs between the DHU loop and TΨC loop is triggered by the codon–anticodon interaction [6,7]. In an attempt to find out whether the latter recognition process induces such a large conformational change in yeast tRNA^{Phe}, we have made an NMR study of the interaction of this tRNA with the oligonucleotide UUCA.

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This tetranucleotide was used as a codon because of its high binding constant [3,8]. To this end the hydrogen-bonded proton spectra as well as the methyl resonances of the tRNA were studied at different temperatures, in the presence and absence of Mg^{2+} and as a function of codon concentration. Under all conditions no loss of hydrogen bonded proton resonances could be detected, meaning that the hydrogen bonds between the DHU and TΨC loops remain intact. At low temperatures the hydrogen-bonded proton resonances of the codon–anticodon complex are visible in the spectra. Comparison of the observed resonance positions with those expected on the basis of ring-current shift calculations indicate that the structure of the anticodon in the codon–anticodon complex differs from the crystal structure of the anticodon in yeast tRNA^{Phe}.

2. Materials and methods

Yeast tRNA^{Phe} was purchased from Boehringer Mannheim; it had an amino acid acceptance of 1400 pmol/A_{260}, as determined by the manufacturer Nicks in the sugar–phosphate backbone will not always impair the chargeability of the tRNA. The material was shown to be completely intact using ^{31}P NMR, by which method terminal and cyclic phosphate groups can be easily detected. Mg^{2+}-free tRNA samples were prepared by dialysing the tRNA as in [9], followed by dialysis against 4 changes of a solution of 1 mM $Na_2S_2O_3$. After dialysis the tRNA was lyophilized; it contained 0.1 mol Mg^{2+}/mol tRNA as determined by

atomic absorption spectrophotometry; tRNA concentrations were calculated using 1 A_{260}/ml = 1.8 μM.

The codon UUCA was synthesized according to the phosphotriester method [10,11]. No impurities could be detected by thin-layer chromatography, high-performance liquid chromatography and ^{31}P NMR; a slight impurity (1%) was, however, detected by ^{1}H NMR, giving rise to a resonance at −1.9 ppm, for which the spectra are corrected. UUCA was used in the Na^+ form; excess salts were removed by Sephadex G-10 gel filtration. UUCA concentrations were calculated from 1 A_{260}/ml = 25.6 μM.

The NMR samples were prepared by dissolving the lyophilized tRNA in 0.2 ml H_2O or D_2O solution containing 100 mM NaCl, 10 mM sodium phosphate at neutral pH and, if indicated in the figure legends, 10 mM $MgCl_2$; the final $Na_2S_2O_3$ concentration was 25 mM. The H_2O samples contained 5% D_2O to lock the field of the NMR spectrometer to the resonance frequency of deuterium.

NMR spectra were recorded on a Bruker 360 MHz NMR spectrometer, operating in the correlation spectroscopy mode; 500–2000 scans of 2 s each were accumulated in a Nicolet BNC 12 computer. Sample temperatures were held constant within 1°C.

The difference spectra were obtained by subtracting the tRNA spectra in the presence and absence of codon, respectively. These spectra were scaled by equating the well resolved low field resonances.

Chemical shifts are indicated in ppm downfield from the methyl resonance of the internal reference 4,4-dimethyl-4-silapentane-1-sulfonate (DSS).

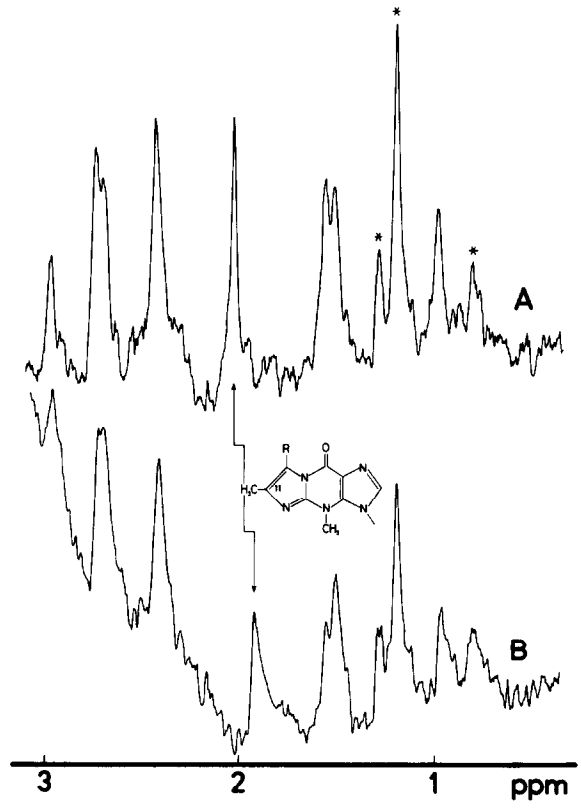

Fig.1. 360 MHz proton NMR spectra of methyl protons in yeast $tRNA^{Phe}$ before (A) and after (B) addition of UUCA. The signal from the C11 methyl group of the Y-base is indicated by arrows. Asterisks indicate impurities. Solution conditions: 0.45 mM tRNA; 0.1 M NaCl; 25 mM $Na_2S_2O_3$; 10 mM phosphate, pH 7; 10 mM Mg^{2+} in D_2O. In spectrum B in addition 6.4 mM UUCA was present. The spectra were recorded at 35°C.

3. Results

The NMR spectra of the methyl protons (0–4 ppm) and of the hydrogen bonded GN1 and UN3 protons (11–15 ppm) of yeast $tRNA^{Phe}$ were recorded at various concentrations of the codon UUCA and at various temperatures.

Figure 1 shows the 360 MHz NMR spectra of the methyl protons of yeast $tRNA^{Phe}$ before and after addition of the codon, recorded at 35°C. The two spectra are virtually identical except for the methyl resonance located at 2.0 ppm, in the absence of codon (fig.1); this resonance shifts upfield upon complexation of the tRNA to UUCA. At this point we shall not attempt a detailed assignment of all the resonances in the spectrum. It suffices to say, that on the basis of NMR studies of anticodon fragments of yeast $tRNA^{Phe}$ and the intact tRNA, the resonance at 2.0 ppm can unambiguously be assigned to the C11 methyl group of the Y-base [13,14] (fig.1), which is located next to the anticodon (fig.2). The resonance position of this methyl group at various concentrations of codon, recorded at 35°C, is given in fig.3. From a non-linear regression analysis of the binding curve in fig.3 we can calculate an equilibrium constant of 1050 l/mol^{-1} and a maximal shift of 0.11 ppm, based on the following reaction:

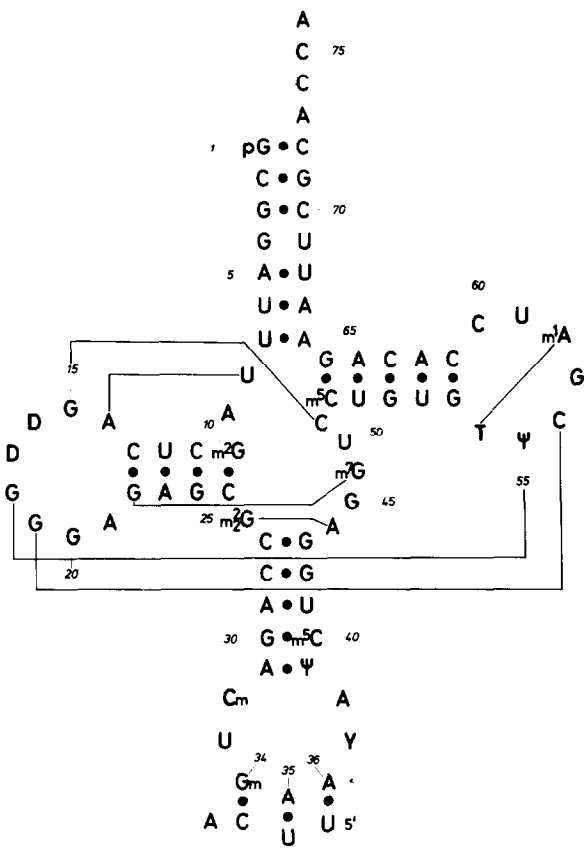

Fig.2. Cloverleaf structure of yeast $tRNA^{Phe}$ [12]. Solid lines indicate tertiary interactions in the tRNA, which may give rise to resonances in the low field NMR spectra. A possible complex between UUCA and yeast $tRNA^{Phe}$ is also indicated. The numbering of the base pairs in the codon–anticodon complex uses the numbering of the residues in the tRNA.

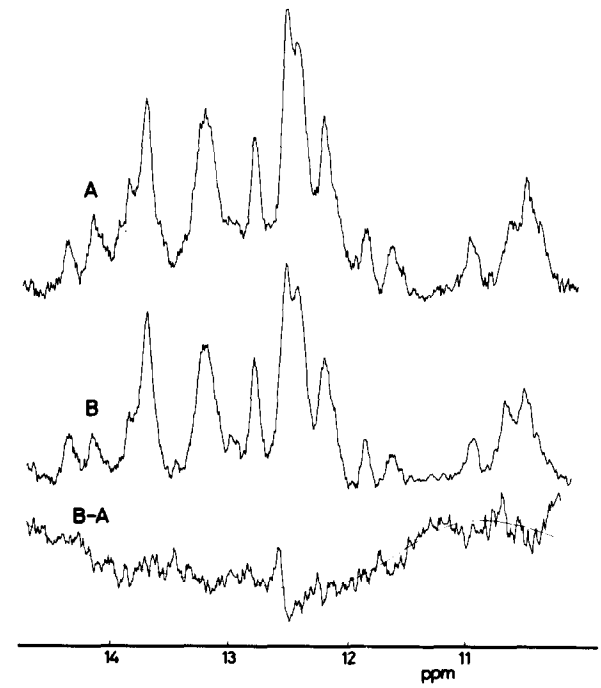

Fig.4. 360 MHz NMR spectra of the hydrogen-bonded protons in yeast $tRNA^{Phe}$ before (A) and after (B) addition of UUCA to conc. 6.1 mM. Spectra were recorded at 35°C. tRNA concentration was 1.26 mM in H_2O. Other solution conditions are indicated in the legend to fig.1, except that no Mg^{2+} was present. The spectrum B–A represents the difference spectrum obtained by subtracting A from B.

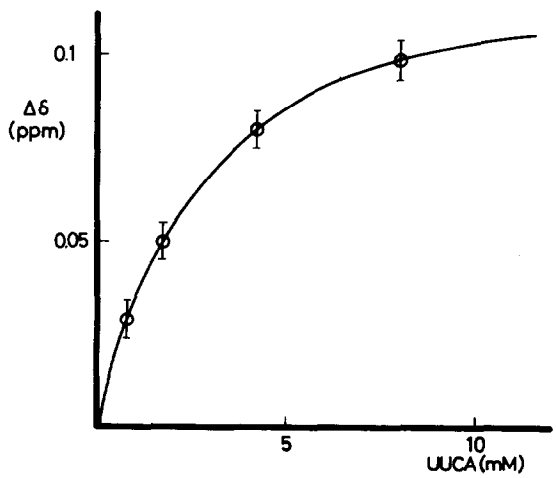

Fig.3. Plot of the changes, Δδ in ppm, in the position of the Y-base C11–CH_3 proton resonance at various concentrations of added UUCA. Spectra were recorded at 35°C; tRNA concentration was 1.4 mM in H_2O. Other solution conditions are indicated in the legend to fig.1, except that no Mg^{2+} was present. The vertical bars indicate the estimated errors in the shift measurements.

$$UUCA + tRNA^{Phe} \leftrightarrows (tRNA^{Phe}\text{–}UUCA) \quad (1)$$

The effect of addition of UUCA on the low field hydrogen bonded proton spectrum of yeast $tRNA^{Phe}$ is shown in fig.4,5.

Figures 4A, 4B represent the tRNA spectra, recorded at 35°C in the absence and presence of codon, respectively. In the latter situation 86% codon binding sites were occupied. The difference between these two spectra is given at the bottom of fig.4. From this it is clear that the most important change between the two spectra occurs around 12.5 ppm. The most likely explanation of this difference is that, upon complexation, a resonance shifts from 12.5–12.6 ppm. In addition a slight change is observable at 10.6 ppm.

At 2°C, at an occupancy of 97% codon binding sites, the GN1 and UN3 protons hydrogen bonded between the codon and tRNA become visible. This is shown in fig.5, where the spectra in the absence (fig.5A) and in the presence (fig.5B) of codon are presented. Again a change is observed around 12.5 ppm analogous to that at 35°C. In addition extra resonance intensity is found at 13.3 ppm and at 11.8 ppm. The signal at 13.3 ppm, which corresponds to about two protons, comes from the hydrogen-bonded UN3 protons, while the signal at 11.8 ppm is thought to be generated by the GC pair. Thus after formation of the codon–anticodon complex the resonances of the hydrogen-bonded protons in two AU and one GC pair become visible in the spectrum. At 11.4 ppm we found a small but reproducible increase in intensity which we cannot as yet explain. The change observed around 10.6 ppm at 35°C is also apparent at 2°C.

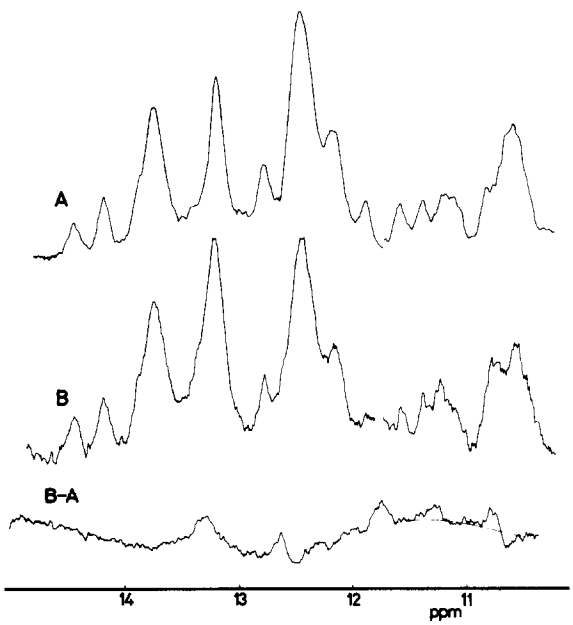

Fig.5. 360 MHz NMR spectra of the hydrogen-bonded protons in yeast $tRNA^{Phe}$, before (A) and after (B) addition of UUCA to a concentration of 1.8 mM. The spectra were recorded at 2°C. tRNA concentration was 1.26 mM in H_2O. Other solution conditions are given in the legend to fig.1, except that no Mg^{2+} was present. The spectrum B–A represents the difference obtained by subtracting A from B. For convenience of representation the right hand part of spectra A and B were subjected to a linear base line correction. The difference spectrum was obtained, however, using the directly measured spectra after they had been cross correlated.

Since the binding of CGAA to the TΨCG sequence of the *E.coli* [6] and yeast $tRNA^{Phe}$–$(pU)_8$ [7] complex was only observed in the presence of at least 8 mM Mg^{2+} our experiments were also carried out with 10 mM Mg^{2+} present in solution. The results were identical to those above for the Mg^{2+}-free solutions.

4. Discussion

The influence of the binding of the codon UUCA on the methyl spectra of yeast $tRNA^{Phe}$, which results only in a shift of the C11 methyl group of the Y-base, demonstrates that the codon is binding to the anticodon loop (fig.1). Measurement of the shift as a function of codon concentration yields a complexation constant of 1050 M^{-1} at 35°C (see section 3). This is in good agreement with the equilibrium constant of 1320 M^{-1}, derived from the data [15]. Since the latter value was derived from data measured at different ionic strength, it should be noted that the binding does not depend very strongly on salt conditions [8,15,16].

The experiments described in this paper were undertaken to find out whether codon–anticodon binding would induce large comformational changes in yeast $tRNA^{Phe}$. It has been suggested [6,7], that the interactions between the TΨC and DHU loop are disrupted upon codon complexation. In this event the following base pairs are expected to be broken: G19–C56; T54–A58; G18–Ψ55 [5]. Although no definitive assignments for the resonances of the GN1 and UN3

protons in these base pairs have been made, we can be reasonably sure that the TN3 proton in T54–A58 resonates from 13.8–14.4 ppm, the GN1 proton in the G19–C56 base pair at about 13.0 ppm and the G18–Ψ55 from 10–12 ppm [17–20].

In the low-field NMR spectra presented in fig.4,5 we see that a resonance at 12.5 ppm shifts slightly downfield after complexation of the tRNA with codon. Since at this position so many resonances overlap, it cannot be assigned to a particular hydrogen-bonded proton in the tRNA molecule. Its position does not coincide with the positions of one of the above-mentioned three tertiary resonances. We wish to mention, however, that the GN1 proton in the G22–G46 base pair (fig.2) resonates at this position [21]; it has been proposed that this base pair plays a keyrole in the unmasking of TΨC and DHU loops [7]. The present results suggest that a major conformational change in the tRNA molecule does not in fact take place as a result of codon–anticodon interaction. This finding is corroborated by the results obtained from the methyl resonance spectra. Since the suggested conformational change should result in a disruption of the A58–T54 base pair (fig.2), an effect on the methyl resonance of T54 is expected analogous to that observed in melting experiments [14,22]. No such change is observed, indicating that the AT pair remains intact after codon–anticodon complex formation.

In the low-field NMR spectra recorded at low temperatures, i.e., at 2°C, the hydrogen bonded proton resonances from the base pairs formed between the codon and the anticodon become visible in the spectrum (fig.5). In total, 4 ring N hydrogen-bonded proton resonances can possibly arise from the interaction between UUCA and the anticodon loop in the spectral region under consideration, one of which will be the GC34 base pair (fig.2). Because of its position, the resonance at 11.8 ppm is assigned to the N_1 proton of G34. This is at such a high field that the 3′-terminal adenine is expected to be stacked favourably upon the GC34 base pair, thereby inducing an appreciable ring-current shift. The resonance at 13.3 ppm then comes from the AU pairs in the codon–anticodon double helix. The resonance has an intensity corresponding to about two protons and it is very likely that one of these comes from the internal AU35 base pair. Interestingly when its resonance position is predicted with ring-current shift calculations [19] in combination with the anticodon structure dictated by the crystal coordinates [1], we find an upfield shift of only 0.1 ppm, leading to a resonance position of 14.4 ppm. This indicates a conformation of the anticodon in the tRNA–codon complex different from that in the crystal structure. We cannot be sure which of the two possible terminal AU base pairs, AU33 or AU36 (fig.2), is formed, i.e., which of the two possible base pairs has such a long lifetime that it can be observed in NMR. We have a preference for the assignment of the remaining resonance intensity at 13.3 ppm to the AU36 base pair since the C11 methyl resonance of the Y-base is shifted upfield, while on the other hand the shift contributions from the Y-base can explain the rather high field position of this AU base pair resonance.

The hydrogen bonded proton resonances from the codon–anticodon complex are not visible in the spectra taken at higher temperatures (see fig.4). This is caused by exchange phenomena. Only at low temperatures is the lifetime of the tRNA–codon complex sufficiently long to allow observation of the GN1 and UN3 protons within the codon–anticodon complex. Considering the following equilibrium:

$$\text{UUCA} + \text{tRNA}^{\text{Phe}} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} (\text{UUCA–tRNA}^{\text{Phe}})$$

k_+ and k_- represent the association rate constant and dissociation rate constant, respectively. These have been determined [8] as a function of temperature by fluorescence-detected T-jump techniques. Under conditions where the broadening of the hydrogen-bonded proton resonances is determined by the lifetime of the complex, the following relation applies:

$$\pi \Delta \upsilon_{1/2} = k_- = \tau^{-1}$$

where τ is the lifetime of the complex and $\Delta\upsilon_{1/2}$ is the line broadening at half height of the resonance. At 2°C, $k_- = 150\ s^{-1}$, yielding a line broadening of about 50 Hz. Under these conditions the resonance will be manifest in the spectra as has been experimentally verified. At higher temperatures k_- rapidly increases, e.g., at 12°C $k_- = 500\ s^{-1}$, yielding a line broadening of 170 Hz. These resonances will be too broad to be detectable among the other resonances; this is in excellent agreement with our observations.

The present results seem to contradict [6,7] in that no major conformational change is observed upon complexation of the tRNA with the codon. It is possible, however, that for a small percentage of the tRNA molecules the DHU–TΨC loop interaction becomes disrupted. This may go undetected in the NMR experiment. It is then possible that the 'open' form of the tRNA molecule in the tRNA–codon complex is substantially stabilized by CGAA. This could explain why appreciable binding of this tetranucleotide to the $tRNA^{Phe}$–$(pU)_8$ complex is found. The use of different codons in both sets of experiments may provide another explanation for the discrepancies observed. These possibilities are presently under investigation.

Acknowledgements

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