HIGH RESOLUTION NMR STUDY OF THE MELTING OF $tRNA^{Phe}_{Yeast}$

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SUMMARY

The 300 MHz NMR spectra of the hydrogen bonded NH ring protons of $tRNA^{Phe}_{Yeast}$ have been measured as a function of temperature. In the presence of Mg^{++} two resonances, one from the Aψ base pair and the other probably from the neighboring base pair, disappear between 56 and 58°C. In the absence of Mg^{++} the DHU stem, the acceptor stem (in particular its AU base pair #6 and #7) and the Aψ base pair in the anticodon stem melt slightly earlier than the other parts of the molecule. Since the DHU stems in $tRNA^{Phe}_{Yeast}$ and $tRNA^{fMet}_{Coli}$ have the same base pairing scheme it is interesting that their melting behavior is entirely different in both molecules. This is discussed in terms of the tertiary structure.

The primary sequence (1) of $tRNA^{Phe}_{Yeast}$ has been known for several years and is homologous with the cloverleaf model of the secondary structure as are all other tRNA sequences. A confirmation of its cloverleaf structure has been obtained lately by NMR studies of the low field hydrogen bonded protons (2) which also gave additional information about base stacking beyond the helical arms (3,4). The crystallographic electron density map at 4 Å resolution is consistent with the cloverleaf secondary structure (5) and reveals many interesting three dimensional features, with more expected soon at still higher resolution. The thermal stability of the secondary and tertiary structure of $tRNA^{Phe}_{Yeast}$ has been studied intensively by two groups with different interpretations, although there has been no exact overlap of solution conditions. In one series (6) of optical studies on Mg^{++} free

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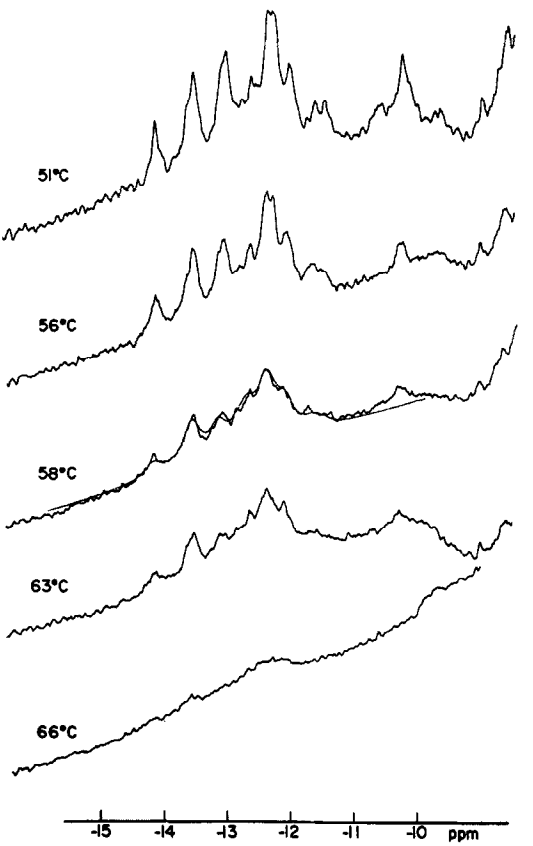

Fig. 1 300 MHz proton NMR spectra of $tRNA^{Phe}_{Yeast}$ as function of temperature. The spectra are the accumulation of about 150 sweeps of 50 seconds each. Volume of the sample was 0.1 ml, $[tRNA^{Phe}_{Yeast}] \approx 2$ mM, $[MgCl_2] = 0.01$ M, $[NaCl] = 0.1$ M, 0.01 M cacodylate buffer at pH 7.0. The horizontal scale gives the position in parts per million (ppm) downfield from DSS.

solutions of $tRNA^{Phe}_{Yeast}$ the equilibrium and relaxation kinetics have been interpreted in terms of a specific sequential melting of the tertiary structure and of the helical arms, although these melting processes were coupled. In another series (7) of optical studies, with and without Mg^{++}, it was suggested that the molecule melts as a whole and that while the melting temperature depends upon the solution conditions, the melting can always be described by a two state model.

Both optical (8) and NMR (9) measurements had agreed that $tRNA^{fMet}_{E.coli}$ in the presence of Mg^{++} melted near 75°C while Cole and Crothers (10) had also shown by relaxation kinetics that in the absence of Mg^{++} $tRNA^{fMet}_{E.coli}$ melted

sequentially. This sequence melting has been confirmed and extended lately by a combined NMR and optical study (11). In this paper we present preliminary NMR measurements of the melting of $tRNA^{Phe}_{Yeast}$ with and without Mg^{++}.

EXPERIMENTAL

$tRNA^{Phe}_{Yeast}$ was purchased from Boehringer-Mannheim and used without further purification. The Mg^{++} free samples were prepared by dialysis against 0.2 M NaCl, 0.01 M cacodylate and 0.01 M EDTA (pH = 7.0) and afterwards concentrated by vacuum dialysis. The Mg^{++} containing solutions were obtained by dissolving the tRNA in the appropriate buffer 0.1 M NaCl, 0.01 M cacodylate and 0.01 M $MgCl_2$ at pH 7.0. The tRNA concentrations were about 50 mg/ml in both cases.

The NMR spectra were obtained on a Varian Associates HR 300, operating in the frequency sweep mode, by accumulating for 2 to 5 hours in Varian 1024 channel analyzer. The shifts are given in ppm with respect to the internal reference DSS. The temperatures are $\pm 1^{\circ}C$.

RESULTS

Figure 1 shows the 300 MHz NMR spectra of low field hydrogen bonded ring NH protons of $tRNA^{Phe}_{Yeast}$ in H_2O solutions with Mg^{++}. The spectrum at $51^{\circ}C$ is very similar to those observed at temperatures down to $20^{\circ}C$ and to those reported previously (2,4). Raising the temperature causes the resonances to broaden (see Fig. 1). In going from $51^{\circ}C$ to $56^{\circ}C$ the broadening is slight, but between 56° and $58^{\circ}C$ it is more pronounced. In this temperature interval two single proton resonances at -13.2 ppm and -12.5 ppm disappear. The dotted line shows a computer simulation of the $58^{\circ}C$ spectrum in which the line positions and intensities are the same as were required to fit the low temperature spectrum, except for the removal of two single proton contributions at -13.2 and -12.5 ppm. In this simulation all of the lines have been broadened by the same amount, i.e. from 35 Hz at $T \leq 51^{\circ}C$ to 100 Hz at $58^{\circ}C$. At $66^{\circ}C$ all the lines are broadened considerably and the integrated intensity has decreased to about one third of its low temperature value.

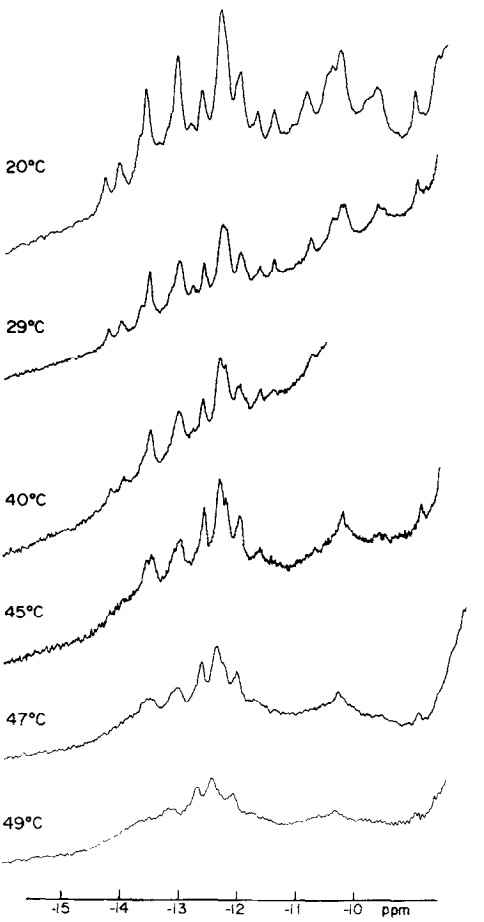

Fig. 2 300 MHz proton NMR spectra of $tRNA^{Phe}_{Yeast}$ as function of temperature. This sample, without Mg^{++}, contained [$tRNA^{Phe}_{Yeast}$] ≈ 2 mM, [NaCl] = 0.2 M, 0.01 M cacodylate buffer at pH 7.0 and 0.01 M EDTA. Spectra are the accumulation of about 75 sweeps of 100 seconds each.

The 300 MHz spectra of $tRNA^{Phe}_{Yeast}$ in 0.2 M NaCl solution without Mg^{++} taken at different temperatures are shown in Fig. 2. The spectra at 20°C and 29°C are identical; they are, however, somewhat different from the low temperature spectra obtained from the solutions containing Mg^{++}. In particular the lowest field resonance, which has previously (3,4) been assigned to AU#6 (see cloverleaf in Fig. 3) is split into two lines. The thermal broadening of the spectra with and without Mg^{++} show definite differences. Without Mg^{++} the AU#6 resonances and the high field component of GC#13 are already broadened at 40°C. Moreover a comparison between 220 MHz

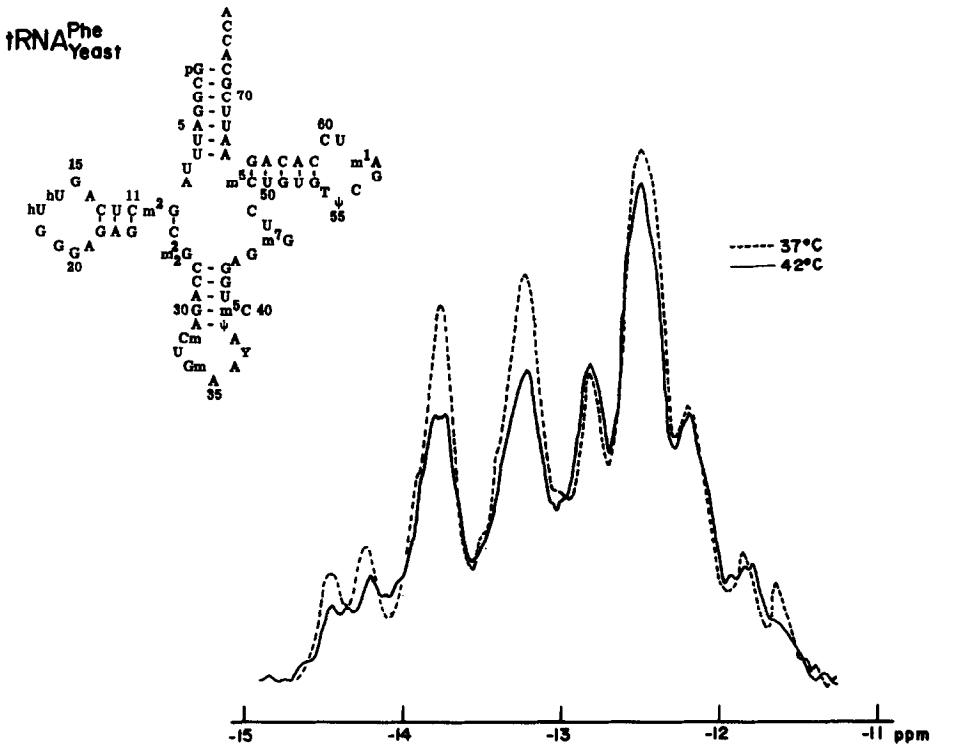

Fig. 3 Comparison of the 39° and 42°C 220 MHz spectrum of $tRNA^{Phe}_{Yeast}$. No Mg^{++}, [NaCl] = 0.2 M, 0.01 M cacodylate buffer and 0.01 M EDTA, pH 7.0.

spectra at 37°C and 42°C reveals a loss of intensity, corresponding to about two protons, mainly in the vicinity of -13.7 and -13.2 ppm (see Fig. 3). Above 42°C the whole spectrum starts to broaden. However this is apparently more pronounced for the part of the spectrum below -12.8 ppm which has previously been assigned primarily to the AU resonances, and for the GC#13 resonance, than for the GC resonances between -12.8 and -12.0 ppm. This behavior is clearly demonstrated in the 47°C and 49°C spectra of Fig. 2. Finally at about 53°C the whole spectrum broadens beyond detection.

DISCUSSION

The broadening and disappearance of the resonances is caused by a decrease in the lifetime of the proton in the helix; as the temperature is raised and the helix becomes more labile the NH proton is transferred from the hydrogen bond in the helix to the nonhydrogen bonded coil from which

state it may exchange with water. Thus the disappearance of the NMR resonances does not necessarily coincide with the melting temperature obtained from optical melting experiments (12).

The results presented here show that $tRNA^{Phe}_{Yeast}$ melts rather abruptly with and without Mg^{++}, i.e. within a temperature region of about 15°C, in contrast to the behavior of $tRNA^{fMet}_{Coli}$ which in the absence of Mg^{++} melts over a temperature range of about 40°C (11). As indicated by the computer simulation of the 58°C spectrum the ring NH proton resonances of $tRNA^{Phe}_{Yeast}$ broaden simultaneously in the presence of Mg^{++} ions, with the exception of the single proton resonances at -13.2 ppm and at -12.5 ppm which disappear at about 55°C. The resonance disappearing at -13.2 ppm is thought to originate from the Aψ#31 residue, which has been shown (4) to melt at lower temperatures in the anticodon half molecule than the other residues in that arm. The single proton resonance disappearing at -12.5 ppm has not been assigned yet with certainty but a likely candidate is the base pair GC#30 adjacent to the Aψ pair which is calculated to fall at -12.3 ppm (3,4). It is interesting to note that Römer et al. (6) have observed a differential melting curve for the Y base fluorescence a few degrees below the UV differential melting curve. Our results, i.e. the early melting of Aψ#31 and possibly of its neighbor GC#30 support their more recent interpretation (13) that the fluorescence behavior of the Y base is connected with the melting of the anticodon stem.

The interpretation of the melting of the $tRNA^{Phe}_{Yeast}$ in absence of Mg^{++} is more involved, but the well resolved resonances at -14.2 and -11.8 ppm previously assigned (4) to AU#6 and GC#13 respectively can be used as markers. Although broader than many other resonances at high temperature, i.e. 49°C, they are still visible indicating that the DHU stem and the acceptor stem still contribute to the NMR spectrum. This observation suggests that these stems (or from the acceptor stem at least the AU containing part, i.e. base pairs AU#7, #6 and probably #5) are melting slightly earlier

than the anticodon and the TψC stem. If we further assume that Aψ#31 melts early as it does in the Mg^{++} containing solution, then this interpretation explains the more pronounced broadening of the part of the spectrum below -12.8 ppm.

In summary, we find that the DHU stem, the acceptor stem (or at least its AU pairs) and base pair Aψ#31 melt slightly earlier than the anticodon stem and the TψC stem. The early melting of Aψ#31 is in accordance with results from optical studies (6,13), where in absence of Mg^{++} it was found, that the quenching and depolarization of the fluorescence of the Y base occurs in the low temperature part of the UV melting curve.

Comparison of the results obtained in this study with the melting behavior of $tRNA^{fMet}_{Coli}$ under about the same solution conditions (11) (i.e. 0.17 M Na^{+} no Mg^{++}) reveals some interesting differences. In particular is noteworthy that the melting of the DHU arm starts ~15°C higher in $tRNA^{Phe}_{Yeast}$ than in $tRNA^{fMet}_{Coli}$, whereas the DHU stems of both molecules have the same base-pairing scheme, except for the reversed polarity with respect to the loop, so that the thermodynamic stability of the isolated DHU hairpins is expected to be about equal (14).

In $tRNA^{Phe}_{Yeast}$ the melting behavior of the DHU arm is as would be expected for the isolated hairpin. This is indicated by the optical studies on the isolated hairpin and the intact $tRNA^{Phe}_{Yeast}$ (4,6) and also by a comparison, between the temperature jump kinetics of the isolated DHU hairpin (in a 0.2 M NaCl solution without Mg^{++}) (15) and the NMR results presented here. On the other hand the combined temperature jump and NMR studies on $tRNA^{fMet}_{Coli}$ showed that the DHU arm does not behave like the isolated hairpin. The kinetic behavior as well as the melting temperature T_m were found to be decidedly anomalous. This was explained by the constraining influence of the tertiary structure (from the interaction between DHU loop and TψC loop) on the DHU arm in $tRNA^{fMet}_{Coli}$, which melts after the DHU stem has melted. However, according to Riesner et al. (13) the tertiary structure in $tRNA^{Phe}_{Yeast}$

melts before the DHU arm and this suggests that in this particular molecule it does not influence the melting of this arm.

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