An NMR study of the exchange rates for protons involved in the secondary and tertiary structure of yeast tRNA\textsubscript{phe}

Paul D. Johnston* and Alfred G. Redfield†

*Department of Biochemistry, Brandeis University, Waltham, MA 02154, and †Departments of Biochemistry and Physics, and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02154, USA

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ABSTRACT

Solvent exchange rates of all the protons of yeast tRNA\textsubscript{phe} resonating in the lowfield NMR region (-11 to -15 ppm from DSS) have been measured by saturation-recovery long-pulse Fourier transform NMR. All these protons in yeast tRNA\textsubscript{phe} are in the fast exchange limit with H\textsubscript{2}O relative to their intrinsic longitudinal relaxation processes. Most rates show very little temperature dependence; however, tertiary base pair protons are preferentially destabilized in the absence of Mg\textsuperscript{2+} at higher temperatures. The measured exchange rates are between 2 and 125 sec\textsuperscript{-1} for a temperature range from 10°C to 45°C and MgCl\textsubscript{2} concentrations between 0 and 15 mM.

INTRODUCTION

Studies by Kearns et al.\textsuperscript{2,3} demonstrated that the ring NH hydrogen-bonded protons from Watson-Crick base pairs of tRNA were in sufficiently slow exchange with solvent H\textsubscript{2}O to be visible by proton NMR at -11 to -15 ppm from DSS. Several studies of these exchange rates have been made by NMR\textsuperscript{4,5} and by tritium out exchange techniques\textsuperscript{6-8}. The present paper reports NMR results which greatly extend these measurements and permit identification of specific rates with specific protons in yeast tRNA\textsubscript{phe}.

More recently, it has been demonstrated that, in addition to a single resonance from each cloverleaf base pair, there also exist from 3 to 7 additional lowfield resonances generated from the tertiary structure of tRNA\textsubscript{phe}\textsuperscript{9-13}. All of these additional base pairs have potential counterparts in the x-ray crystal structure of yeast tRNA\textsubscript{phe}\textsuperscript{14-16}. Römer et al.\textsuperscript{17} and Riesner et al.\textsuperscript{18} have shown by U.V. differential melting experiments that the tertiary structure in yeast tRNA\textsubscript{phe} is preferentially destabilized
to thermal melting under conditions of no Mg$^{++}$ and low ionic strength. In order to detect this preferential melting behavior of the tertiary resonances, we have studied the exchange properties of the lowfield NH resonances under conditions of high, intermediate and zero levels of MgCl$_2$. The NMR studies were done using a long-pulse FT-NMR technique which allows the observation of proton spectra in the presence of 95% H$_2$O. This technique has been described in detail elsewhere; a 2-1-4 (~250 μsec) pulse was used to flip over the spins of the lowfield protons without flipping over the H$_2$O spins. The tRNA proton signals were then processed by Fourier transform techniques without serious interference from the large H$_2$O signal.

**MATERIALS AND METHODS**

Yeast tRNA$^{\text{Phe}}$ was either purchased from Boehringer Mannheim Ltd. and further purified by DEAE-Sephadex chromatography or was purified from unfractionated tRNA by two-times BD-cellulose chromatography followed by DEAE-Sephadex chromatography. The final material was assayed according to B.R. Reid et al. using partially purified phe-tRNA-synthetase, and all tRNA$^{\text{Phe}}$ accepted at least 1.7 nmols phe/A258 unit.

All NMR tRNA samples were dialyzed twice against six liters of 10 mM EDTA and then dialyzed similarly against distilled H$_2$O and lyophilized. Samples containing 2.7 mgs of tRNA were dissolved in 90 μl of buffer for the following NMR buffer solutions: (1) 10 mM Na cacodylate, pH 7.0, 1 mM EDTA, 0.1 M NaCl; (2) 10 mM Na cacodylate, pH 7.0, 15 mM MgCl$_2$, 0.1 M NaCl. Samples containing 5.0 mgs of tRNA were dissolved in 200 μl of buffer for the following NMR buffer solutions: (1) 10 mM Na cacodylate, pH 7.0, 10 mM EDTA, 0.1 M NaCl; (2) 10 mM Na cacodylate, pH 7.0, 1.5 mM MgCl$_2$, 7 mM EDTA, 0.1 M NaCl. All samples were then extensively dialyzed against the NMR buffer solutions in a microdialysis cell (except the 1 mM EDTA sample). The 90 μl NMR spectra were taken using a micro-cell (Wilmad). The 200 μl NMR spectra were taken with a 5 mm NMR tube using a teflon vortex plug (Wilmad). The 1 mM EDTA sample was not dialyzed into the NMR buffer solution and certainly contains some Mg$^{++}$; unfortunately the exact amount is unknown. Chemical shifts were measured relative to solvent H$_2$O.
and converted to approximate DSS reference by subtracting 4.8 ppm. All spectra were obtained on the LDB-270 spectrometer, which was built by A. Redfield and S. Kunz using a Bruker 270 MHz magnet and 5 mm probe, and Nova 1220 computer\textsuperscript{19,20}. The 2-1-4 pulse\textsuperscript{19} was used for all observation pulses. Transfer of saturation experiments were done using a 0.5 sec saturation pulse, and saturation recovery experiments were done using a 0.1 - 0.4 sec saturation pulse. The power of all preirradiation pulses was set to be just sufficient to nearly saturate the irradiated resonance with a short (~5 msec) delay between the preirradiation and the observation pulse. Either a spoil or a pulse droop\textsuperscript{19} was used to help decrease the unwanted H$\textsubscript{2}$O signal which was stimulated by the preirradiation pulse. We could measure the H$\textsubscript{2}$O nuclear magnetization directly after preirradiation by means of a standard long observation pulse. An attenuator was inserted early in the receiver system for these measurements, to prevent electronic overload. We could thereby verify that the H$\textsubscript{2}$O protons were not affected by preirradiation in the downfield region, and that the H$\textsubscript{2}$O protons were completely saturated when preirradiation was at the water frequency.

RESULTS

A. Transfer of Saturation

In order to use saturation recovery as a measure of exchange rates of exchangeable protons, it is necessary to show that these protons are in fast exchange with solvent H$\textsubscript{2}$O relative to their intrinsic longitudinal relaxation processes. A transfer of saturation experiment\textsuperscript{24} demonstrating the fast exchange between H$\textsubscript{2}$O and the ring nitrogen protons is presented in Fig. 1. A long, selective preirradiation pulse (0.5 sec) was applied at the H$\textsubscript{2}$O resonance frequency, spectrum (a). The pulse frequency was then moved away from H$\textsubscript{2}$O toward the lowfield tRNA resonance frequencies in a series of steps. We verified quantitatively that the intensities of the downfield peaks were proportional to the H$\textsubscript{2}$O intensity for the same preirradiation conditions.

Since the experimental relaxation times measured as described below are, in some cases, quite long (~500 ms) then the longitudinal relaxation times for the ring NH must be unusually
Figure 1. Transfer of saturation between H$_2$O and yeast tRNA$_{\text{phe}}$ in buffer containing 0.1 M NaCl, 1.5 mM MgCl$_2$, 7 mM EDTA, 10 mM sodium cacodylate, pH 7.0, 35°C. Preirradiation from H$_2$O resonance frequency = (a) 0 Hz, (b) 210 Hz, (c) 310 Hz, (d) no preirradiation.

long, on the order of seconds. The possibility exists that relaxation occurs via a nuclear Overhauser effect (NOE) between the amino protons and ring NH and that the transfer of saturation with water is then an indirect transfer via the amino protons. This possibility has been tested by looking for NOE effects between the amino protons and ring NH; none were found. This explanation for the transfer of saturation experiments therefore seems unlikely.

The transfer of saturation was tested for each solution condition discussed in this paper. The results were essentially identical to those shown in Fig. 1 at 15°, 23° and 45°C. Therefore, we assume that the saturation-recovery behavior described below is dominated by chemical exchange processes.

B. Saturation-Recovery

An example of a saturation-recovery experiment is shown in Fig. 2. A selective preirradiation pulse was applied at -14.2
Figure 2. Saturation-recovery measurement of the -14.4 to -14.0 ppm spectral region of yeast tRNA in 10 mM EDTA, no Mg recorded at 30°C. The preirradiation pulse was applied at -14.2 ppm with the delay time between preirradiation and observation pulses given in milliseconds.
ppm in order to saturate the region from -14.0 to -14.4 ppm without saturation of H$_2$O. Spectra were recorded with different values of delay $\tau$ between the preirradiation and the observation pulses. The recovery of individual peaks with longer $\tau$ occurs almost entirely because of in-exchange from the unsaturated solvent pool of protons as shown by the transfer of saturation experiment (Fig. 1). The data was fit to one, or occasionally two, exponentially recovering curves in the usual way, and the first order rate constant(s) of these exponentials are reported below as exchange rates without correction for possible intrinsic relaxation.

Spectra of yeast tRNA$^{\text{phe}}$ under the three solution conditions explored by saturation recovery are shown in Fig. 3. The most notable spectral changes when lowering the MgCl$_2$ concentration are the shifts around -12.3 ppm, -12.6 to -13.0 ppm and the splitting of the resonance at -14.4 ppm. Note that the 1 mM EDTA sample certainly contains a low level of Mg$^{++}$ (see METHODS), and it is essentially identical to spectra obtained in 0.5 mM MgCl$_2$ (G.T. Robillard, private communication).

C. Temperature dependence of exchange: Mg$^{++}$ containing samples (15 mM MgCl$_2$, 1 mM EDTA, trace Mg$^{++}$)

The proton exchange rates for these two samples generally showed weak or no temperature dependence (see Table I). Fig. 4 (a and b) shows a typical Arrhenius plot for the 15 mM MgCl$_2$ sample and for the 1 mM EDTA (trace Mg$^{++}$) sample. We do not have an interpretation for the lack of temperature dependence for the exchange process under these solution conditions. The exchange mechanism may be a low energy process requiring no destacking of bases or may be a coupled stacking/destacking which requires no net energy.

Interesting exceptions to this behavior are: a) In the high-Mg$^{++}$ sample, many protons exhibited a small negative enthalpy of activation in their exchange rates; of these, the proton resonating at -12.9 ppm is certainly experimentally significant, since its rate decreased five-fold between 23°C and 45°C (Table I; 23°C data not shown in Table I). The exchange rate of the peak at -12.9 ppm, in the 1 mM EDTA sample, also showed a decrease in its rate with an increase in temperature between 36°C and 45°C.
Figure 3. The experimental 270 MHz proton NMR spectra of yeast tRNA\textsubscript{1} in a buffer containing 0.1 M NaCl, 10 mM sodium cacodylate pH 7.0 and the given concentration of MgCl\textsubscript{2}, or EDTA. The spectrum marked 1 mM EDTA is thought to contain trace amounts of Mg\textsuperscript{2+} (see text).

This suggests a minor change in the average structure such that this proton (which is probably a tertiary proton, see below) is more exposed at lower temperatures; b) In trace Mg\textsuperscript{2+} (1 mM EDTA sample), two protons resonating at -13.35 and -11.75 ppm showed relatively large temperature dependence (roughly 3-fold increase, from 36°C to 45°C) in their exchange rates (see Table I). These are also likely to be tertiary base pair protons.

For both samples, the integral of the spectrum from -11 to -15 ppm indicated 26 ± 2 protons observable between 15°C and
Table I. Temperature dependence of exchange. The temperature
dependence of the observed exchange shown for the three solution
conditions discussed in the text. Additional data (not shown)
was taken at 15°, 23°, and 50°C for the 15 mM MgCl₂ sample and
at 30° and 50°C for the 1 mM EDTA sample. Two rates are given
by a slash and indicates biphasic rate behavior.

<table>
<thead>
<tr>
<th>Proton Intensity</th>
<th>10 mM EDTA Temperature (°C)</th>
<th>1 mM EDTA Temperature (°C)</th>
<th>15 mM MgCl₂ Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10° 30° 36° 39° 42° Exchange Rates (Sec⁻¹)</td>
<td>36° 45° Exchange Rates (Sec⁻¹)</td>
<td>Peak ppm from DSS</td>
</tr>
<tr>
<td>1</td>
<td>-14.4 4 15 19 29 125</td>
<td>2 -14.4 9 10</td>
<td>2 -14.4 7 5</td>
</tr>
<tr>
<td>1</td>
<td>-14.2 5 16 20 26 65</td>
<td>1 -13.9 6 5</td>
<td>1 -13.9 11 7</td>
</tr>
<tr>
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<td>-14.0 7 33 24 33 --</td>
<td>2 -13.8 5 8</td>
<td>3 -13.8 8 7</td>
</tr>
<tr>
<td>1</td>
<td>-13.9 - 12 18 -- 38</td>
<td>1 -13.7 5 --</td>
<td>1 -13.4 13 14</td>
</tr>
<tr>
<td>2</td>
<td>-13.8 8 5 8 -- 7</td>
<td>2 -13.8 5 8</td>
<td>1 -13.7 8 --</td>
</tr>
<tr>
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<td>4 -13.2 3 5</td>
<td>4 -13.25 8 10</td>
</tr>
<tr>
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<td>1 -12.9 4 2</td>
<td>2 -12.9 9 5</td>
</tr>
<tr>
<td>1</td>
<td>-12.8 5 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td>1 -12.7 4 4</td>
<td>1 -12.7 10 4</td>
</tr>
<tr>
<td>7</td>
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<td>7 -12.5 4 8</td>
<td>7 -12.5 11 5</td>
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</tr>
<tr>
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<td>1 -11.8 20 8</td>
</tr>
<tr>
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<td>-11.6 - 8 15 -- 125</td>
<td>1 -11.6 4 9</td>
<td>1 -11.7 13 7</td>
</tr>
</tbody>
</table>
Figure 4. The Arrhenius plot for the early melting transition in yeast tRNA^phe. Plotted are the lowfield protons’ (-11 to -15 ppm) observed exchange rates: (a) peak at -13.8 ppm, • 15 mM MgCl₂, ● 1 mM EDTA, ▲ 10 mM EDTA; (b) peak at -14.4 ppm, ■ 15 mM MgCl₂, ● 1 mM EDTA; (c) 10 mM EDTA, △ peak at -14.4 ppm, ▲ peak at -14.2 ppm.

45°C; therefore no melting has taken place in this temperature interval.

D. Temperature dependence of Exchange : 10 mM EDTA, (no Mg^{2+})

For this sample, there appear to be two classes of protons primarily distinguished by their rate behavior above 36°C. One class showed essentially no temperature dependence in exchange rates over the entire temperature range studied (10° to 42°C), and members of this class are probably secondary base pair protons. The protons in this class resonate at -13.8, -13.2, -12.7
and -12.2 ppm (see Table I). The rate behavior of these protons is illustrated by the Arrhenius plot, Fig. 4 (a), shown for the protons resonating at -13.8 ppm.

The other class of protons exhibited strong temperature-dependent exchange rates. These protons generally fall into two subclasses: (1) Those which show normal Arrhenius behavior from 30°C through 42°C (resonances at -13.9, -13.35, -12.95, -12.5 and -11.75 ppm), and (2) those which show an upward break in their Arrhenius plots above 36°C (resonance at -14.4, -14.2 and -11.6 ppm and possibly some protons in subclass (1)). The resonance at -12.5 ppm showed biphasic rate behavior for which the higher rate represented about two protons and the slower rate represented about five protons (see Table I). An Arrhenius plot for resonances at -14.4 ppm and -14.2 ppm is presented in Fig. 4 (c). The low temperature segment of the plot yields an activation energy of about 10-15 Kcal/mole for each peak and the higher temperature segment about 50-90 Kcal/mole. The activation energies for the protons in subclass (1) and the resonance at -11.6 ppm are about 20-40 Kcal/mole.

One other proton at -14.0 ppm showed peculiar behavior; between 10° and 30°C its rate increased five-fold, and at higher temperatures (30° to 39°C) its exchange rate, although relatively high, did not show marked temperature dependence.

Several of the peaks appeared to first lose integrated intensity without broadening as the temperature was raised and then broadened at higher temperatures. The spectrum integral from -11 to -15 ppm decreased from about 25 ± 2 protons at 36°C to about 19 ± 2 protons at 42°C, suggesting a slow conversion to a partially melted conformation.

DISCUSSION

These results show that the exchange rates of the ring NH protons are sensitive to structural changes in yeast \textit{tRNA}^{\text{phe}}. The rates observed are in agreement with those measured by Campbell \textit{et al.} using a slightly different transfer of saturation technique, and the rates are consistent with a D$_2$O mixing experiment as observed by NMR. Our data cover a lower temperature range and are taken at a different ionic strength than the line broadening studies of \textit{E. coli} \textit{tRNA}^{\text{fmet}} by D. M. Crothers,
et al.\textsuperscript{26} and of yeast tRNA\textsuperscript{phe} by C. W. Hilbers et al.\textsuperscript{27}. However, our results are consistent with their data in that, for example, the most downfield resonances (below -14.0 ppm) show fast high-temperature kinetics.

In order to discuss these observed exchange rates in terms of structural events such as melting, it is necessary to separate such a structural event from the actual chemical exchange. The following simple kinetic model is useful, although not necessarily complete:

\[
\begin{align*}
\text{closed} & \xrightleftharpoons[k_{\text{cl}}]{k_{\text{op}}} \text{open} \xrightarrow{k_{\text{ch}}} \text{exchange} \\
& \quad \text{with } \text{H}_2\text{O}
\end{align*}
\]

where the H\textsubscript{2}O exchange may be catalyzed by OH or buffer. This model has been thoroughly discussed for polynucleotides\textsuperscript{28} and for tRNA\textsuperscript{26}. Eigen's theory of proton transfer permits one to calculate an estimate of the exchange rate for UN\textsubscript{3}H and GN\textsubscript{1}H protons\textsuperscript{29}. The calculation gives a chemical exchange rate of 6 x 10\textsuperscript{-4} sec\textsuperscript{-1} for both of these protons at 10 mM cacodylate, pH 7.0, (assuming a pk for UN\textsubscript{3}H and GN\textsubscript{1}H of 9.5). To compare this rate to the structural rate constants, \(k_{\text{op}}\) and \(k_{\text{cl}}\), one can use the relaxation rate of 10\textsuperscript{2} sec\textsuperscript{-1} determined for the tertiary structure melt by optical methods\textsuperscript{18,34}. Since the latter rate is much less than the free base exchange rate, our observations would yield \(k_{\text{op}}\) if this model is applicable to our sample and buffer conditions.

We have found a preferential destabilization as a function of temperature for protons resonating at -14.4, -14.2, -14.0, -13.90, -13.35, -12.95, -12.5, -11.75 and -11.6 ppm in the absence of Mg\textsuperscript{2+} (10 mM EDTA). Of these, the six protons at -14.4, -14.2, -13.35, -12.95, -11.75 and -11.6 ppm, all have life-times of 10 to 15 msecs at 42°C. Only a small amount of Mg\textsuperscript{2+} (sample labeled 1 mM EDTA, see METHODS) is needed to stabilize all but two of these protons (-13.35 and -11.75 ppm) and to produce observable changes in the spectrum.

It is our hypothesis that the above six Mg\textsuperscript{2+}-sensitive resonances represent tertiary base pair interactions or secondary base pair interactions whose melting behavior is strongly coupled to tertiary structure. Resonances at -14.4 ppm and
-14.2 ppm (at -14.4 ppm in MgCl₂) have been previously assigned to tertiary interactions U8-A14 and T54-m¹A58 in yeast tRNA^phe on the basis of ring current calculations using the x-ray coordinates of yeast tRNA^phe, 12,30. Oxidation of S4-U8-A14 to U8-A14 in E. coli tRNA^fmet, 10 and E. coli tRNA^val, 23 has been used to assign U8-A14 at -14.4 ppm. Based on our exchange data, we would say that the resonance at -14.4 ppm (in solutions containing MgCl₂) is the tertiary base pairs U8-A14 and T54-m¹A58 with one being shifted to -14.2 ppm in the absence of Mg++. In three published accounts of ring current calculations 12,30,31 the tertiary Watson-Crick base pair G19-C56 has been assigned to the region from -12.7 ppm to -12.95 ppm. On the basis of exchange behavior we can now tentatively assign the resonance at -12.95 ppm (in 10 mM EDTA) to be the tertiary base pair G19-C56.

This data also provides experimental evidence to support ring current calculations 12,30 which place the tertiary base pair G15-C48 at -11.75 ppm (in 10 mM EDTA). We find the assignment of CG 28 (an internal secondary base pair of acceptor stem) at -11.6 ppm 12,30,31 to be inconsistent with our experimental results. No other protons in this stem (especially AU 29 and AU 31) showed the marked temperature-dependent rate seen for the proton at -11.6 ppm. The resonance at -11.6 ppm follows the melting of the second part of the tertiary structure in that its rate behavior is similar to that observed for U8-A14 and T54-m¹A58. This behavior suggests that the resonance is due to a tertiary interaction. Although speculative, possible candidates are the tertiary base pairs involving a ring nitrogen proton to oxygen hydrogen bond such as G18-755 14 or U33 32. Another possibility is that the resonance represents GC 13 as has been proposed in the literature 32. This would also be consistent with our melting scheme (see discussion below). Several tertiary base pairs have previously been assigned at -13.8 ppm 12,31. Again we find this inconsistent with our results because of the lack of temperature dependence for the protons at -13.8 ppm, (see Fig. 4 (a)). On the basis of our exchange results we tentatively assign the resonance at -13.35 and -12.5 ppm to be one or the other tertiary base pair m²G26-A44 or G22-m⁷G46.

The exchange rate data in high Mg++ showed no interpretable...
signs of breakdown of tertiary or secondary structure as the temperature is raised from 12°C to 45°C. However, data with low Mg and with no Mg indicate structural perturbations and melting of tertiary structure. In a rigorously Mg ++ free solution (10 mM EDTA, 0.1 M NaCl), there appears to be an early transition observable below 36°C, followed by a widespread breakdown of tertiary structure above this temperature and an incipient loss of part of the secondary structure, probably the DHU stem. These changes may indicate sections of the molecule which are also flexible in the more biologically relevant high Mg ++ state. We will now discuss this in more detail.

Between 30°C and 36°C, three protons in the Mg ++ -free sample (resonances at -11.75, -12.95 and -13.35 ppm identified as tertiary base pairs C48-G15, G19-C56 and either G22-m7G46 or m2G26-A44 respectively) show similar temperature-dependent rates with activation energies of 20 to 40 Kcal/mole. There is no apparent change in the spectrum integral, and this behavior probably indicates an incipient fast conversion to a state of higher enthalpy in which these base pair protons exchange more rapidly. Because there are no gross changes in the spectra, this state exists only a small fraction of the time. This transition appears to be partially present in the 1 mM EDTA (trace Mg ++ ) sample as indicated by the rate behavior of the protons at -13.35 and -11.75 ppm. Since this transition basically involves outside base pairs (G19-C56 and C48-G15)14 (see Fig. 5) it may be similar to the fraying reaction observed for the terminal base pairs of short DNA helices.

Over this same temperature range, in the rigorously Mg ++ -free sample, several other protons start to show a lesser temperature dependence in their exchange rate which is absent in the presence of Mg ++ . They include both tertiary and non-tertiary protons. The most notable changes are reflected by the AU resonance at -13.9 ppm, the proton at -11.6 ppm, the tertiary base pair at -12.5 ppm (G22-m7G46 or m2G26-A44) and additional non-tertiary base pairs at -12.5 and the low temperature segment (below 36°C) of the rate profile for the tertiary base pairs U8-A14 and T54-m1A58 (resonances at -14.4 and -14.2 ppm) (Fig. 4 (c)).

Above 36°C, in the Mg-free sample, there is a break in the
Arrhenius plot for the protons resonating at -14.2 and -14.4 ppm (T54-m1A58 and U8-A14) signalling a second, more cooperative process with an activation energy of 50-90 Kcal/mole. Some other protons also appear to show an upward break in their rates over this temperature range. Most notable of these is the resonance at -11.6 ppm and possibly the resonances at -13.35, -12.95 and -12.5 ppm. By 42°C, five protons show a similar rate of about 100 sec⁻¹ and there is appreciable loss of integrated intensity, suggesting a melting transition at a rate of 100 sec⁻¹ to a state in which the tertiary protons exchange more rapidly than 1000 sec⁻¹. However, the tertiary base pair at -12.5 ppm is still largely intact as judged by the exchange rates for the resonance at -12.5 ppm.

Turning to probable secondary protons, we note the broad resonance at -14.0 ppm in the Mg²⁺-free sample which has not previously been reported, and we speculate that it is AU 5, shifted from -13.8 ppm in Mg²⁺-containing samples by the solution conditions in the Mg²⁺-free sample. This probably reflects structural changes around GU 4.

The remaining AU proton which shows a temperature-dependent
rate in the Mg\textsuperscript{++}-free sample resonates at \(-13.9\) ppm. We use this resonance to determine which arm of the secondary structure has begun to become destabilized in the temperature range of this study. The acceptor, T\textsubscript{YC} and anticodon stems all contain more than one AU or AV base pair and if any of these had started melting there would be more than one temperature-dependent AU proton. Thus, we tentatively assign the \(-13.9\) ppm resonance to AU 12, which is consistent with ring-current calculations\textsuperscript{12}. The temperature-dependent rate observed for the composite resonance at \(-12.5\) ppm may represent in part the GC protons of the DHU stem with the possibility that \(-11.6\) ppm is GC 13. Thus, we predict that the next segment of the molecule to melt would be the DHU stem plus the tertiary base pair at \(-12.5\) ppm. It is reasonable, on the basis of the x-ray structure, that the DHU stem should be sensitive to the loss of tertiary structure and possibly be subject to early melting because of its short length and because of its considerable involvement in tertiary interactions to the T\textsubscript{YC} loop and extra loop (see Fig. 5). This interpretation disagrees with that of Römer and co-workers who infer that the acceptor and anticodon helices melt with the residual tertiary base pair(s) while the T\textsubscript{YC} and DHU helices are the last to melt\textsuperscript{34,35,36}.

To summarize, in a Mg\textsuperscript{++}-free solution we observe an early transition involving the outside tertiary base pairs G19-C56, G15-C48 and a proton at \(-13.35\) ppm. This is followed by a highly cooperative melt of most tertiary structure above 36°C. Part of the tertiary structure is still intact at this point (proton at \(-12.5\) ppm) and we feel this tertiary base pair is beginning to melt with some less well defined secondary structure, probably DHU stem. In terms of the model of equation 1, at the highest temperature we may be observing \(k_{op}\) for some part of the tertiary structure. At lower temperatures, for the protons which show temperature dependence, \(k_{ch}\) may be rapid and rate-determining, modulated by a fast equilibrium to some "open" state which is not unfolded but from which exchange is more rapid than from the low-temperature state. Further experiments such as the effect of buffer concentration on these rates\textsuperscript{28} may help determine exact mechanisms of exchange.
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REFERENCES

1. Abbreviations used: DEAE - diethylaminoethyl; BD - benzoyl-diethylamino ethyl; UV - ultraviolet; NOE - nuclear Overhauser effect; yeast tRNA_phe - Baker's yeast phanylalanine transfer ribonucleic acid; A - adenosine; C - cytidine; G - guanosine; U - uridine; F - pseudouridine; T - ribothymidine; m^G - N^1 methylguanosine; DHU - dihydouridine; m^G - N^1,2 dimethylguanosine; m^A - 1-methyladenosine; m^C - 5-methylcytidine; m^7G - 7-methylguanosine; DSS - 2,2-dimethyl-2-silapentane-5-sulfonate; EDTA - ethylenediamine tetra acetic acid; FT-NMR - Fourier Transform-NMR; S - U - 4-thiouridine.
