Determination of the angle between the anticodon and aminoacyl acceptor stems of yeast phenylalanyl tRNA in solution

(RNA structure/protein biosynthesis/transient electric birefringence/gel electrophoresis/transcription)

MARISA W. FRIEDERICH, FRANK-ULRICH GAST*, ELSI VACANO, AND PAUL J. HAGERMAN†

Department of Biochemistry, Biophysics, and Genetics, B-121 University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262

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ABSTRACT A principal feature of the crystal structures of tRNAs is an L-shaped tertiary conformation in which the aminoacyl acceptor stem and the anticodon stem are approximately perpendicular. However, the anticodon–acceptor interstem angle has not been precisely quantified in solution for any tRNA. Such a determination would represent an important test of the predicted global conformation of tRNAs in solution. To this end, we have constructed a yeast tRNA\textsuperscript{Phe} heteroduplex RNA molecule in which the anticodon and acceptor stems of the tRNA have each been extended by approximately 70 base pairs. A comparison of the rotational decay times of the heteroduplex molecule and a linear control yields an interstem angle of 89 ± 4° in 4 mM magnesium chloride/100 μM spermine hydrochloride, essentially identical to the corresponding angle observed in the crystal under similar buffer and temperature conditions. The current approach is applicable to the study of a wide variety of RNA molecules that possess elements of nonhelical structure.

In the nearly two decades that have elapsed since the final refinements were reported for the crystal structure of yeast tRNA\textsuperscript{Phe} (1–3), a wealth of data has accrued regarding tRNA structure in solution. These data generally support the notion that local structures in the crystal and in solution are quite similar. However, key features of the global structure of yeast tRNA\textsuperscript{Phe} (and of tRNAs in general) have not been amenable to direct experimental confirmation. In particular, the angle between the two arms of the “L” predicted to be about 90° in the crystal (2), has been difficult to define with precision in solution. For example, fluorescence resonance energy transfer (FRET) measurements (4, 5) using fluorophores attached to the 3′ terminus and to the anticodon loops of yeast tRNA\textsuperscript{Phe} and Escherichia coli tRNA\textsuperscript{Glu} have yielded interfluorophore distances of 40–60 Å for tRNA\textsuperscript{Phe} (4) and >65 Å for tRNA\textsuperscript{Glu} (5). Using the framework of yeast tRNA\textsuperscript{Phe}, these distance uncertainties would represent interarm angles in the range of 30°–60° for tRNA\textsuperscript{Phe} and >70° for tRNA\textsuperscript{Glu}. In all likelihood, those results are not so much indicative of different interstem angles for the two tRNAs as they are reflective of markedly reduced precision for transfer distances that approach the upper limit for the dye pairs employed.

An alternative approach for the determination of interhelix angles, one that is not subject to the distance limitations imposed on the otherwise sensitive fluorescence resonance energy transfer measurements, involves the application of transient electric birefringence (TEB; ref. 6) to determine the apparent bend angle created by a nonhelical element (e.g., bulge, junction, etc.) following its insertion into the center of an otherwise duplex RNA helix. In a TEB experiment, the RNA molecules are oriented by a brief (~1 μsec) electric field pulse. This partial orientation renders the solution optically anisotropic, as reflected in the polarization-dependence of the refractive index of the solution (birefringence). Following removal of the field, rotational randomization of the RNA molecules is accompanied by the decay of birefringence, the rate of decay being a sensitive measure of the overall extension of the molecules in solution.

By analogy to the electrophoretic and hydrodynamic properties of curved/bent DNA (7–10), bent RNA molecules are expected to manifest both reduced electrophoretic mobilities and reduced rotational reorientation times relative to linear duplex RNA molecules having the same contour lengths. We have demonstrated previously that TEB measurements are capable of yielding precise estimates of the interhelix angles for both branched and bent RNA molecules (11–14). For one such system—namely, the self-cleaving “hammerhead” RNA domain (11)—all three interhelix angles, both precleavage and postcleavage, have been determined for an extended, all-RNA molecule that remains fully active in self-cleavage.

The approach taken for the current investigation is exactly analogous to the previous TEB studies of branched and/or bent RNA. Specifically, an extended yeast tRNA\textsuperscript{Phe} heteroduplex molecule (designated E[tRNA\textsuperscript{Phe}] heteroduplex), along with its linear duplex RNA counterpart, have been assembled according to the scheme presented in Fig. 1. TEB measurements of this pair of constructs have yielded an acceptor–anticodon interstem angle that is nearly identical to the corresponding angle in the crystal.

MATERIALS AND METHODS

Preparation of RNA Expression Plasmids. DNA oligomers corresponding in sequence to the 5′ half (\textit{"{d}dU}) in Fig. 2; nucleotides 1–31) and the 3′ half (\textquoteleft{}TsC\textquoteright{} in Fig. 2; nucleotides 39–72) of yeast tRNA\textsuperscript{Phe} (plus short flanking sequences) were synthesized along with their complements and were inserted into the \textit{Hind}III cloning sites of plasmids pGJ122A and pGJ122B, respectively. These two parent plasmids are pGEM7Zf(+) derivatives in which the sequence between residues 2692 and 72 of pGEM has been replaced by a T7 promoter and a 136-nucleotide template, the latter terminated by a \textit{Sma} I cleavage site (Fig. 2). The promoter/template segments were originally described in plasmids pFU3A and pFU3B, respectively (16), and were moved into pGEM-derivative plasmids for improved yield. The template exists in two orientations, A and B, so that transcription from both templates yields fully complementary RNAs (excluding any cloned insertions). The appropriate derivative plasmids, designated pGJ122A9 and pGJ122B11, were identified by sequencing and were maintained in DH5\textsubscript{a}F\textsuperscript{−} cells (Promega).

Abbreviations: TEB, transient electric birefringence; E[tRNA\textsuperscript{Phe}], extended tRNA\textsuperscript{Phe}; °E, relative electrophoretic mobility.

*Present address: Max-Planck Institut für Biochemie, Abteilung Viroidforschung, D-82143 Planegg-Martinsried, Germany.
†To whom reprint requests should be addressed.
RESULTS

Gel Electrophoretic Behavior of the E[RNA\textsuperscript{Phe}] Heteroduplex Molecule. An example of the electrophoretic behavior of the E[RNA\textsuperscript{Phe}] heteroduplex is presented in Fig. 4. It is evident in Fig. 4 \textit{Left} that the mobility of the construct is dramatically reduced (μ\textsubscript{rel} = 0.69; 6% polyacrylamide gel). Moreover, μ\textsubscript{rel} is found to decrease dramatically with increasing gel concentration (Fig. 4 \textit{Right}). These two observations are entirely consistent with the gel behavior of other RNA species possessing centrally located bends (11–14, 20–22), although the presence of increased flexibility in the tRNA core cannot be ruled out. It should also be noted that the μ\textsubscript{rel} of the E[RNA\textsuperscript{Phe}] heteroduplex is essentially independent of temperature below 40°C, suggesting that the angle of the \textit{L} is not changing to any significant degree over this temperature range. This last observation is consistent with our observation that the E[RNA\textsuperscript{Phe}] heteroduplex undergoes specific, lead-induced cleavage [between residues U17 and G18 in the dhU loop (23–25)] at temperatures up to \textordmasculine{sim}50°C (M.W.F. and P.J.H., unpublished results).

In preparation for the TEB studies of the E[RNA\textsuperscript{Phe}] heteroduplex construct, the dependence of μ\textsubscript{rel} on Mg\textsuperscript{2+} concentration was examined. The mobility of the heteroduplex molecule gradually decreased as the Mg\textsuperscript{2+} concentration was lowered below \textordmasculine{sim}4 mM, decreasing by \textordmasculine{sim}20–25% as the Mg\textsuperscript{2+} concentration approached 50 μM (Fig. 5). Further reductions in Mg\textsuperscript{2+} concentration were accompanied by a significant increase in μ\textsubscript{rel}. Although this latter effect has not been investigated in detail, it may reflect the loss of tertiary structure expected to occur following the removal of divalent cations.
indicates that the curve; the parameters function of the construction are given in the text. Sequence elements bounded by the inverted triangles correspond to the yeast tRNA{Phe} sequence. "T4C" and "dhU," sequences of the 3' half (nucleotides 1–31) and the 5' half (nucleotides 39–72), respectively, of yeast tRNA{Phe}; A and B, orientation of the T7 template; is, the HindIII insertion site; S, Sma I cleavage site; aa and ac, acceptor and anticodon stems, respectively.

**Determination of the Acceptor–Anticodon Interstem Angle of the E[tRNA{Phe}] Heteroduplex Molecule by TEB.** To quantify the anticodon–acceptor interstem angle, TEB measurements were performed on the E[tRNA{Phe}] heteroduplex and its linear counterpart at 3.5°C in the presence of 4 mM Mg^{2+} and 100 μM spermine. The results of a set of these measurements are presented in Fig. 6. Analysis of the terminal birefringence decay times for five separate measurements each for the heteroduplex and linear species yielded values of $\tau_{\text{heteroduplex}} = 1.70 \pm 0.08 \mu s$ and $\tau_{\text{linear}} = 3.15 \pm 0.01 \mu s$ (3.5°C values), for a resultant experimental ratio, $\tau_{\text{heteroduplex}}/\tau_{\text{linear}}$, of 0.54 ± 0.03. Comparison of the experimental $\tau$ ratio to the computed curve (Fig. 3) yielded an apparent angle between the anticodon and

**Fig. 2.** Outline of the approach followed in constructing the heteroduplex RNA molecules. Details of the construction are given in the text. Sequence elements bounded by the inverted triangles correspond to the yeast tRNA{Phe} sequence. "T4C" and "dhU," sequences of the 3' half (nucleotides 1–31) and the 5' half (nucleotides 39–72), respectively, of yeast tRNA{Phe}; A and B, orientation of the T7 template; is, the HindIII insertion site; S, Sma I cleavage site; aa and ac, acceptor and anticodon stems, respectively.

**Fig. 3.** Computed $\tau$-ratio plot for a 174-bp RNA molecule as a function of the central bend angle, $\theta$. The plot of $\tau/\tau_{180}$ vs. $\theta$ was generated by using the program DIFFROT (10, 14, 18), with helix parameters as specified in the text. The horizontal dashed line indicates that experimental $\tau$ ratios are extended from the ordinate to the curve; a further vertical extension of the curve to the abscissa yields the interstem angle.

**Fig. 4.** Electrophoretic behavior of the E[tRNA{Phe}] heteroduplex relative to the 174-bp linear control. (Left) Positions on 6% polyacrylamide gel (25°C) of the heteroduplex molecule and the linear control. Lanes: a, 174-bp linear RNA control with a relative mobility, $\mu_{\text{rel}}$, of 1.0; b, E[tRNA{Phe}] heteroduplex with a $\mu_{\text{rel}}$ of 0.69. (Right) $\mu_{\text{rel}}$ plotted as a function of gel concentration. Most gels were run at 25°C (B) with TEB buffer containing 4 mM MgCl$_2$. Additional 6% gels were run at 8°C (A) and 40°C (C). Buffers were recirculated during gel runs.
acceptor stems of $89 \pm 4^\circ$, a value that is entirely consistent with the value of $82^\circ$ reported for the equivalent interstem angle in the crystal (2). It should be noted that whereas some increase in flexibility in the tRNA core cannot be ruled out at present, an increase in flexibility for bends in the $90^\circ$ range is unlikely to significantly bias the apparent angle (ref. 26; discussed in more detail in ref. 14).

DISCUSSION

The current investigation has provided evidence that yeast tRNA$^{Phe}$ in solution possesses the same interarm angle as the crystal form of the molecule under similar conditions of buffer and temperature. However, it should be noted that the current investigation has employed RNA transcripts that lack the specific posttranscriptional modifications that are present in the native tRNA. While the absence of such modifications could potentially alter the conformation of the low-temperature structure, the greatest effect of the elimination of modifications in the core of tRNA$^{Phe}$ is probably a net decrease in the stability of the molecule (27), not a substantial change in its structure. This contention is based on the fact that the unmodified yeast tRNA$^{Phe}$ transcript is capable of being correctly aminocylated by its cognate synthetase, which requires both anticodon and acceptor stem contacts, suggesting that the interstem angle has not changed significantly in the unmodified transcript (27). Moreover, the tRNA$^{Phe}$ transcript can undergo specific, lead-induced cleavage of the dU loop (between residues U17 and G18) (28), indicating that coordination between the TψC and dU loops is quite similar in the modified and unmodified forms. As noted above, we have observed the same site-specific (lead-induced) cleavage in the dU loop of the extended tRNA$^{Phe}$ heteroduplex. In this regard, it would be of interest to study the yeast tRNA$^{Ap}$ species, where there is more solution evidence for differences between the modified and unmodified forms (29).

Quite apart from the specific issue of the interarm angle in yeast tRNA$^{Phe}$, the current study represents an approach for addressing two fundamental questions regarding tRNA structure: (i) to what extent do the interarm angles vary among the tRNAs and (ii) what tertiary interactions are specifically required for the maintenance of the L-shaped conformation? Regarding the first question, results from the tRNA crystals (30, 31) and from tRNA synthetase cocrystals (32, 33) suggest that the range of angles is relatively narrow, a conclusion supported by studies of mischarging within classes of E. coli amber suppressors (34). Regarding the second question, the published tRNA crystal structures make clear predictions as to which tertiary interactions are likely to stabilize the global conformations of tRNAs. The combined gel/TEB approach should facilitate a direct examination of those predictions.

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