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Intensity and anisotropy decays of the Wye base of yeast tRNA^{Phe} as measured by frequency-domain fluorometry

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The intensity and anisotropy decays of Wye base fluorescence from yeast tRNA^{Phe} were determined by frequency-domain fluorometry. The intensity decay is at least a double exponential in the presence and absence of Mg^{2+}, but the multi-exponential character of the decay is more pronounced in the absence of Mg^{2+}. The anisotropy decay displays components due to overall tRNA rotational diffusion and to local torsional motions. The amplitude of the local motion is decreased 2-fold by the presence of Mg^{2+}. The results are broadly consistent with a more homogeneous environment for the Wye base in the presence of Mg^{2+}.

1. Introduction

The existence of multiple conformations for tRNA has been proposed to explain experimental observations and biological function. As studied by fluorescence spectroscopy, the most extensive data for tRNAs have been obtained using either extrinsic labels such as eithidium bromide or proflavin, or the intrinsic label wyebutine (Wye) at position 37 in the anticodon loop of yeast tRNA^{Phe} [1]. The fluorescence of the Wye base from tRNA^{Phe} was found to display multiple decay times, which were attributed to a family of equilibrium conformers [1]. The multiple-conformer model has also been used to explain *T*-jump kinetic data [2] and NMR data [3]. The multiple conformers are not unique for yeast tRNA^{Phe}, as was shown by recent NMR studies on *E. coli* tRNA^{Phe} [4]. The proportion of the conformers is apparently altered by the magnesium concentration, which offers a facile method to study the conformers.

We used the technique of frequency-domain fluorometry [5–8] to examine the effects of magnesium on the intensity and anisotropy decays of the Wye base in yeast tRNA^{Phe}. Earlier time-resolved measurements of tRNA^{Phe} revealed a dominant single decay time [9]. Our results are in general agreement. However, we recovered two decay times for Wye in yeast tRNA^{Phe}, and two correlation times for the anisotropy decay. The intensity and anisotropy decays were both found to be sensitive to the presence of magnesium.

2. Materials and methods

Yeast tRNA^{Phe} was purchased from Boehringer Mannheim. The sample was dissolved in 10 mM Tris, 100 mM KCl (pH 7.4) or the same buffer plus 10 mM $MgCl_2$. The concentration of tRNA used for these measurements was 8×10^{-6} M. The amount of magnesium in the lyophilized material

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is variable and often not removable by dialysis. The complete removal of magnesium from tRNA is difficult, the resultant tRNA is fragile and the backbone is easily cleaved in samples which have undergone extensive treatment to remove magnesium. Dialysis will remove all but 0.1–0.5 magnesium per tRNA (S. Seifried and B. Wells, unpublished data). As the sample to be used in these experiments had to be stable for days we did not employ extensive dialysis. The lifetime and steady-state measurements of the samples indicated a considerable amount of magnesium. At the time of the measurement we added 0.5 mM EDTA to complex the residual magnesium. Since the fluorescence spectral properties of the Wye base were altered by EDTA we believe the EDTA was effective in removing Mg^{2+}.

The frequency-domain fluorometer was described previously [7], as were the methods for analysis of the intensity [6] and anisotropy decays [8]. The excitation source was an HeCd laser at 325 nm. Emission was observed through a liquid filter (2 mm) of 1 M $NaNO_2$ to eliminate scattered light. For intensity decay measurements the excitation was vertically polarized, and the emission at 54.7° from the vertical. These polarization conditions eliminate the effect of rotational diffusion on the intensity decays. All phase and modulation measurements were relative to POPOP in ethanol using a reference lifetime of 1.31 ns [10]. The absorbance of POPOP was less than 0.1 at the excitation wavelength. Preliminary data were obtained on the SLM 4800 with only three frequencies, which did not provide adequate resolution of the multi-exponential decays.

3. Results

Extensive studies have indicated that the Wye base fluorescence intensity approximately doubles with added magnesium (ref. 11 and references cited therein). The quantum yield increases from 0.04 to 0.09, so changes in the lifetime are expected. The fluorescence intensity of the putative low-magnesium sample was found to be too high. Hence, EDTA was added to 0.5 mM.

The frequency response for $tRNA^{Phe}$ with added EDTA is shown in fig. 1. The dashed line represents the best single decay time fit to the data (○), and the solid line the best double-exponential fit. Evidently, the data cannot be accounted for by a single decay time. The advantages of a wide range of modulation frequencies is evident from the deviations (lower panels of fig. 1). For the single-component fit the deviations (○) are systematic and much larger than the experimental uncertainties of 0.2 degrees of phase and 0.002 in modulation. In contrast, the deviations are small and random for the double-exponential fit (○), indicating that this model is adequate to account for the data. We do not regard the modest decrease in χ^2_R from 2.3 to 1.8 to demonstrate three decay times (table 1). Hence, the decay is dominated by a 5.9 ns component, and 23% of the total intensity has a decay time near 1.7 ns.

The effect of chelating the residual magnesium is seen from the first two entries in table 1. Relative to the sample without added EDTA, the

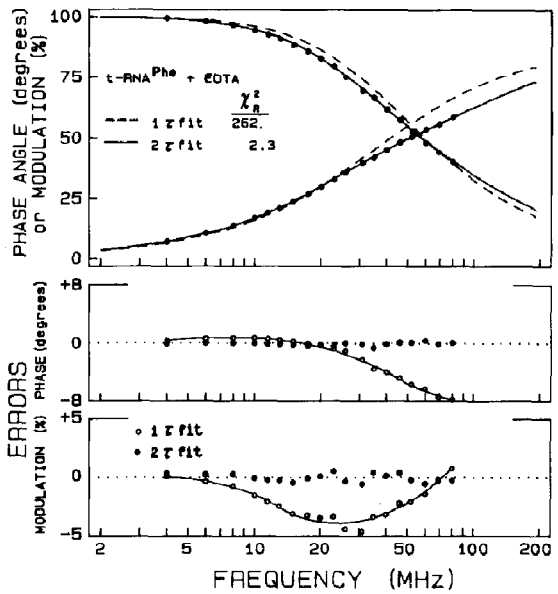

Fig. 1. Frequency response of yeast $tRNA^{Phe}$ Wye base fluorescence, in the presence of 0.5 mM EDTA. The upper panel shows the data (●), and the best one (------) and two (———) decay time fits. The lower panel shows the deviations from the calculated fit for one (○) and two (●) decay times.

Table 1

Intensity [a] decay parameters for the Wye base in yeast $tRNA^{Phe}$

Sample conditions	τ_i (ns)	α_i	f_i	χ^2_R
10 mM Tris, 100 mM KCl, 25 °C (pH 7.4)	5.4	1.0	1.0	57.7 [d]
	2.5 (0.10) [b]	0.31	0.15	0.96
	6.1 (0.05)	0.69	0.85	
	2.1 (0.7) [c]	0.24	0.11	0.97
	5.4 (4.0)	0.56	0.60	
	7.1 (4.0)	0.20	0.29	
As above plus 0.5 mM EDTA	4.6	1.0	1.0	261.8
	1.7 (0.07)	0.50	0.23	
	5.9 (0.06)	0.50	0.77	2.3
	0.6 (0.7)	0.28	0.05	1.8
	2.6 (0.7)	0.35	0.27	
	6.2 (0.2)	0.37	0.68	
10 mM Tris, 100 mM KCl, 10 mM $MgCl_2$	5.6	1.0	1.0	11.1
	2.7 (0.4)	0.16	0.08	1.4
	6.0 (0.1)	0.84	0.92	
	0.6 (0.7)	0.11	0.01	0.86
	5.6 (0.2)	0.87	0.95	
	12.4 (0.5)	0.02	0.04	

[a] $I(t) = \Sigma\alpha_i e^{-t/\tau_i}$, $f_i = \alpha_i\tau_i / \Sigma\alpha_i\tau_i$, χ^2_R = reduced chi square.

[b] The values in parentheses are the estimated uncertainties [16].

[c] These decay times are equivalent to being within the uncertainty estimated from the usual assumptions of nonlinear least squares [16].

[d] The values of χ^2_R were calculated using $\sigma_p = 0.2°$ as the uncertainty in the phase values and $\sigma_m = 0.002$ as the uncertainty in the modulation.

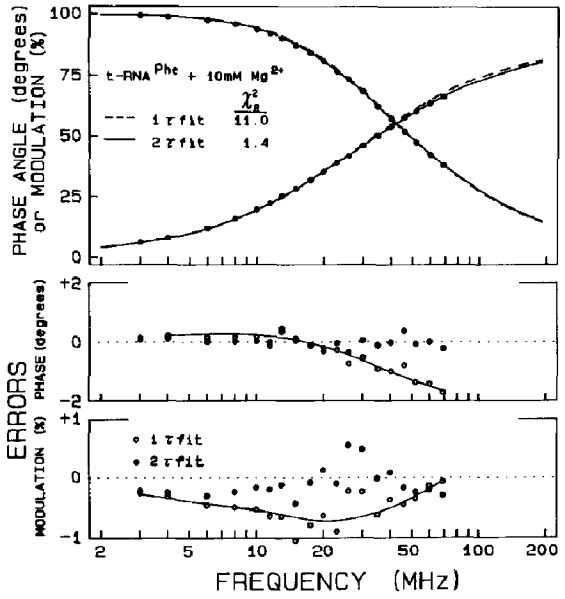

Fig. 2. Frequency response of yeast $tRNA^{Phe}$ Wye base fluorescence, in the presence of 10 mM Mg^{2+}. See fig. 1 for details.

magnesium-free form of $tRNA^{Phe}$ displays an increased amplitude for the shorter lived component, and a decay which is more heterogeneous. This is seen by comparing the values of χ^2_R for single decay time fits, which increase from 58 to 262 upon addition of EDTA. In contrast, addition of magnesium results in a more homogeneous decay. This is seen by the smaller value of χ^2_R for the one decay time fit (table 1), and from the smaller deviations (fig. 2). Evidently, the decay is predominately a single exponential in the presence of magnesium, but a short component is still required to fit the data. If the multiple lifetimes are diagnostic of conformational populations, then for different solution conditions with different proportions of the conformers, one might expect the same τ_i values but varying α_i values. Different amounts (α) of nearly the same lifetimes do occur for the two-component fits, which are the fits we believe best describe the data. For all three samples the small decrease in χ^2_R for the three-component fits, and the increased uncertainties in the recovered decay times, indicate that the three-component model is not well supported by the data. The similarity of the two decay times in the presence and absence of magnesium, and the sensitivity of the amplitudes to magnesium, suggest a conformational origin for the two decay times. These results suggest that the Wye base exists in two environments; in one the lifetime is quite short (2 ns) and the intensity low; in the other, the lifetime is 6 ns with a larger intensity. In the presence of EDTA (low magnesium) the two environments occur in equal amounts ($\alpha_1 \simeq \alpha_2$), whereas the addition of magnesium increases the amount of the 6 ns form.

We also examined the anisotropy decays of the Wye base in the presence and absence of mag-

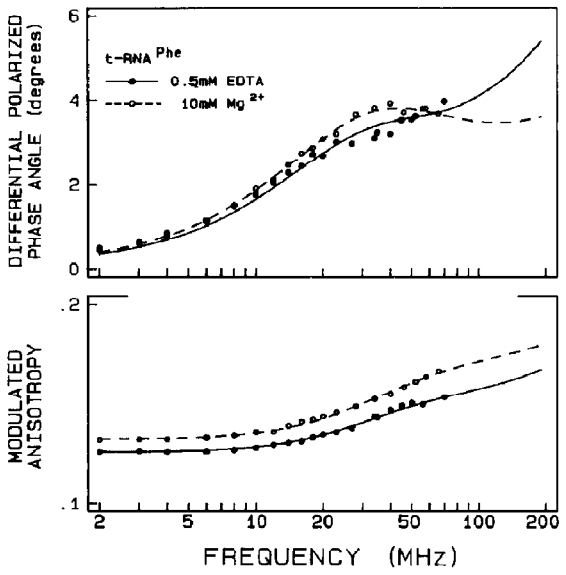

Fig. 3. Anisotropy decay of the Wye base fluorescence in the absence (EDTA) and presence of $MgCl_2$.

nesium. The frequency-domain anisotropy data show peaks or shoulders near 30 MHz, which is expected for overall rotational diffusion of tRNA with a correlation time near 18 ns. It should be noted that the precise frequencies of the phase angle maxima are dependent upon the decay law of the sample, and the extent of local motions of the fluorophore. Additionally, the phase angles continue to increase at higher modulation frequencies, which indicates faster motions of the Wye base. In the absence of magnesium, the amplitude of the global motion is decreased, and the high-frequency (> 30 MHz) phase angles increased, relative to the sample with added magnesium (fig. 3). Alternatively stated, 77% of the anisotropy decay in the presence of magnesium is due to overall rotational diffusion and 23% is due to local motions (table 2). Removal of magnesium results in a more rapid anisotropy decay in which 42% of the anisotropy decays by torsional motions of the Wye base.

Table 2

Anisotropy [a] decay parameters for the Wye base of yeast $tRNA^{Phe}$

Sample conditions	$r_0 g_i$	θ_i (ns)	χ^2_R
10 mM Tris, 100 mM KCl, 0.5 mM EDTA	0.17	12.5	8.7 [b]
	0.11	0.3	
	0.15	18.5	1.5
10 mM Tris, 100 mM KCl, 10 mM $MgCl_2$	0.18	14.9	2.3
	0.05	0.4	
	0.17	17.4	0.5

[a] $r(t) = \Sigma r_0 g_i e^{-t/\theta_i}$.

[b] $\sigma_p = 0.1°$ and $\sigma_m = 0.005$.

4. Discussion

The double-exponential Wye base lifetimes are in agreement with the interpretation of similar data for ethidium bromide-labeled tRNA. The question arises as to what are the molecular structures of the two forms. A prevalent interpretation has been the 3′-stack vs. the 5′-stack for the bases in the anticodon loop [1]. The 3′-stack of bases 34, 35, 36, 37 and 38 occurs in the crystal structure which contains magnesium and so the predominant form found at high magnesium is assumed to be a 3′-stack [12,13]. The other form could then be a 5′-stack but other evidence tends to favor some other form at low magnesium. The NMR evidence cited in ref. 11 argues against a 5′-stack as do chemical modification studies [14]. The nonhydrogen-bonded bases in the anticodon loop are bases 32–38, the 3′-stack having bases 32 and 33 out of the stack; presumably a 5′-stack would result in base 33 being stacked between bases 32 and 34. There is a major change in chemical accessibility of bases 33 and 34 as a result of changing the ionic strength. The two bases show large amounts of modification in 1 mM magnesium ($\mu = 0.1$) and low modification in 10 mM magnesium ($\mu = 0.25$). If a 5′-stack exists in low magnesium one would expect just the opposite results. A 5′-stack at high magnesium is consistent with the chemical modification data but not with the fluorescence data. Under conditions of very high ionic strength, 500 mM KCl and no added magnesium, Clore et al. [15] found a 3′-stack with hydrogen bonds connecting C_{32} to Wye_{37}, and base 33 was hydrogen-bonded to bases 36 and 35 [15]. The low-mag-

nesium form has bases 33 and 34 (chemical modification data) as well as base 37 (fluorescence data) relatively exposed to solvent at 0.1 M ionic strength. At high ionic strength or upon addition of magnesium, these bases are rigidly held in place (NMR, chemical modification and fluorescence). A consistent explanation is that the anticodon loop is quite flexible with low magnesium and an ionic strength of 0.1 M. The bases are freely twisting and tilting, allowing greater exposure to the solvent. Upon addition of magnesium or at very high ionic strength, the loop is constrained. This model is consistent with the anisotropy decays, which show a decreased amplitude for Wye base torsional motions in the presence of magnesium. The magnesium binds inside the loop, being directly coordinated to phosphate 37 and through water to bases 37–39, 31 and 32; this perfects the stack of 37 between 38 and 39 resulting in the enhanced fluorescence and in tethering the entire loop makes 33 and 34 less accessible to modifying reagents. The high ionic strength in the isolated anticodon loop also ties down the loop resulting in the hydrogen-bonding network seen in the NMR study [15]. The two conformers (as exemplified by the anticodon loop) are thus a loose, flexible loop and a constrained, taut loop. As these conformers are in equilibrium, the amount of each conformer varies so the detection of a pure form is not favored.

Acknowledgements

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