Internal Motions in Yeast Phenylalanine Transfer RNA from ^{13}C NMR Relaxation Rates of Modified Base Methyl Groups: A Model-Free Approach†

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ABSTRACT: Internal motions at specific locations through yeast phenylalanine tRNA were measured by using nucleic acid biosynthetically enriched in ^{13}C at modified base methyl groups. Carbon NMR spectra of isotopically enriched $tRNA^{Phe}$ reveal 12 individual peaks for 13 of the 14 methyl groups known to be present. The two methyls of N^2,N^2-dimethylguanosine (m_2^2G-26) have indistinguishable resonances, whereas the fourteenth methyl bound to ring carbon-11 of the hypermodified nucleoside 3′ adjacent to the anticodon, wyosine (Y-37), does not come from the [*methyl*-^{13}C]methionine substrate. Assignments to individual nucleosides within the tRNA were made on the basis of chemical shifts of the mononucleosides [Agris, P. F., Kovacs, S. A. H., Smith, C., Kopper, R. A., & Schmidt, P. G. (1983) *Biochemistry 22*, 1402–1408; Smith, C., Schmidt, P. G., Petsch, J., & Agris, P. F. (1985) *Biochemistry 24*, 1434–1440] and correlation of ^{13}C resonances with proton NMR chemical shifts via two-dimensional heteronuclear proton–carbon correlation spectroscopy [Agris, P. F., Sierzputowska-Gracz, H., & Smith, C. (1986) *Biochemistry 25*, 5126–5131]. Values of ^{13}C longitudinal relaxation (T_1) and the nuclear Overhauser enhancements (NOE) were determined at 22.5, 75.5, and 118 MHz for $tRNA^{Phe}$ in a physiological buffer solution with 10 mM $MgCl_2$, at 22 °C. These data were used to extract two physical parameters that define the system with regard to fast internal motion: the generalized order parameters (S^2) and effective correlation times (τ_e) for internal motion of the C–H internuclear vectors. For all methyl groups the generalized order parameter varied from 0.057 to 0.108, compared with the value of 0.111 predicted for a rapidly spinning methyl group rigidly mounted on a spherical macromolecule. Values of τ_e ranged from 4 to 16 ps, generally shorter times than measured in other work for amino acid methyl groups in several proteins. Somewhat surprising was the finding that the two methyl esters terminating the Y-37 side chain have order parameters similar to those of other methyls in tRNA and only 25% less than that for a methyl directly bonded to the base. The side chain may have stabilizing interactions within the anticodon loop that prevent large amplitude, segmental motion. Values of the order parameter S^2_{ax} calculated for motion of methyl group axes (rotation factored out) varied over the range 0.51–0.97. If the motion of the axis were described as random diffusion within a cone, these values would correspond to cone semiangles of 37° and 11°, respectively. The most restricted group was found to be the ring-bound N^3-methyl of Y-37 in the anticodon loop. The least restricted methyl is on the ring of ribothymidine-54. In general, the anticodon loop methyls were more highly motion restricted than other $tRNA^{Phe}$ methyl groups.

Function of transfer RNA ultimately depends on dynamics of structural elements. Knowledge of tRNA dynamics is improving (Rigler & Wintermayer, 1983; Prabhakaran et al., 1985; Leroy et al., 1985), but many data are still contradictory, come from experiments using entrinsic probes, or come from studies under nonphysiological solution conditions. Carbon-13 NMR relaxation time measurements offer a way to gauge amplitudes, rates of motion, and information on internal mobility in biological macromolecules without perturbing the structure and under completely natural solution conditions (London, 1980). However, the 1.1 atom % natural abundance of carbon-13 precludes the study of dynamics in macromolecules. The required sensitivity is obtained through ^{13}C enrichment of the molecule.

The present paper is the first attempt to interpret methyl group ^{13}C relaxation time data for biosynthetically enriched yeast tRNA. A key advantage of [^{13}C]methyl data compared to those of the ^{31}P NMR of phosphodiesters (Gorenstein & Goldfield, 1982), the enriched ^{13}C of ribose (Schmidt et al., 1983), adenosine or cytidine base carbons (Schmidt et al., 1980), or the uracil base carbons (Schmidt et al., 1980; Olsen et al., 1982) is the secure assignments of all [^{13}C]methyl resonances to individually modified bases throughout the structure.

Transfer RNA from yeast or *Escherichia coli* can be enriched in ^{13}C at selected loci by using [*methyl*-^{13}C]methionine for the incorporation in vivo of [^{13}C]methyl groups during synthesis of modified nucleosides (Agris et al., 1975, 1983; Tompson et al., 1979). This technique permits sufficient signal-to-noise ratios to be obtained with the necessarily limited tRNA quantities, and it provides a sound starting point for peak assignments. We had previously reported ^{13}C relaxation times of methyl groups in four *E. coli* tRNAs (Kopper et al., 1983), three of which had three methyls and the fourth only two.

Yeast $tRNA^{Phe}$ has fourteen methyl groups, distributed throughout most of the molecule. Thirteen of these were

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isotopically enriched to 70% by growing *Saccharomyces cerevisae* in the presence of [*methyl*-^{13}C]methionine. Carbon-13 NMR spectra of the purified yeast tRNA^{Phe} revealed at least one resolved peak for each ^{13}C-enriched modified nucleoside (Figure 1). Assignments were made earlier for all peaks (Smith et al., 1985; Agris et al., 1986).

Because methyl carbons have directly bonded protons, the NMR longitudinal and transverse relaxation times (T_1 and T_2, respectively) are generally dominated by the ^{13}C–^{1}H dipole–dipole interaction, at least at lower NMR frequencies. Interpretation of T_1 and T_2 data, along with the nuclear Overhauser effect (NOE), can yield values for the amplitudes and rates of reorientation of the C–H internuclear vectors (London, 1980). Some procedural choices are available when there is internal motion as well as overall motion of the macromolecule, as in the case of a methyl group on tRNA. The motion may be modeled as a series of specified internal reorientations superimposed on overall motion (London, 1980) or without appeal to a model, using instead a generalized order parameter to collect the net internal motions of a relaxing nucleus (Lipari & Szabo, 1982a,b). In the work reported here we examined tRNA^{Phe} internal motions using the latter "model-free" approach. Our investigation indicates that only data collected within a specific range of NMR frequencies are valid for the model-free approach. The results suggest that the formalism may not be accurate for tRNA motion at the highest NMR frequencies employed (75.5 and 118 MHz for ^{13}C). Lower field data (22.5 MHz) gave consistent and presumably more accurate results.

Experimental Procedures

Materials. Transfer RNA^{Phe} was isolated from [*methyl*-^{13}C]methionine-fed cultures of *S. cerevisae* met-trp- (RH762) and purified as previously described (Agris et al., 1983; Smith et al., 1985).

NMR Spectroscopy. Transfer RNA samples were prepared for NMR by extensive dialysis against ethylenediaminetetraacetic acid (EDTA) in double-distilled water and then against highly purified water. Samples were concentrated by vacuum, exchanged twice with D_2O, and placed in phosphate-buffered saline (PBS) of pH 7.2 in D_2O (10 mM $MgCl_2$, 15 mM KH_2PO_4, 10 mM Na_2HPO_4, 100 mM NaCl). Final concentrations of tRNA were approximately 1.3 mM tRNA (0.6 mL). Spectra were obtained at 22.5 MHz (^{13}C) with a JEOL FX-90Q with a 5-mm NMR probe, at 75.5 MHz with a Nicolet NT 300 with a 5-mm single-frequency ^{13}C probe, and at 118 MHz with a Nicolet NT 470 with a 10-mm broad-band probe and a 0.45-mL sample insert. Longitudinal relaxation, T_1, measurements were made at 22.5 MHz by using the inversion–recovery method and at 75.5 and 118 MHz by using progressive saturation. NOE measurements were made with gated broad-band ^{1}H decoupling for the null measurements and continuous broad-band decoupling for expression of NOE. The computer cycled back and forth between sets of free induction decays to maintain a relatively constant temperature. Delay times in the gated decoupling experiment were carefully checked to allow full relaxation of magnetization to its equilibrium level, making note of the observation that long ^{1}H T_1 values may indicate extended wait times (Jelinski et al., 1980). Transverse relaxation times (T_2) were estimated from line widths.

Theory. One of two general approaches is usually pursued for analysis of NMR relaxation data in terms of internal molecular dynamics. The motion can be modeled as a series of specified types of internal reorientation superimposed on overall rotational diffusion. To the extent that there is sufficient data, the model can be tested for its validity, and parameters of the motion can be extracted (London, 1980; Lipari & Szabo, 1981; Keepers & James, 1982). The second approach treats the data in a model-free format where all internal motion is described by only two values, a generalized order parameter, S^2, and a correlation time (Lipari & Szabo, 1982a,b). We and others have used the specific model approach for nucleic acid motion using ^{13}C (Schmidt et al., 1983) and ^{31}P (Bolton & James, 1980) relaxation times and NOEs.

We chose to pursue the model-free analysis in order to gain some insight into motion in tRNA^{Phe} without presupposing a particular mode or modes of that motion. Lipari and Szabo (1982a) based their analysis in part on the premise that overall motion is described by a single correlation time, τ_m. The analysis further assumes that overall macromolecule reorientation is in the nonextreme narrowing region ($\omega\tau_m > 1$) and that all internal motion is extreme narrowing ($\omega\tau_{int} < 1$), where ω is the NMR frequency in radians per second. Lipari and Szabo constructed a simple expression for the spectral density containing only τ_m; a generalized order parameter, S^2; and a value for internal motion, the internal correlation time (τ_e).

Using this spectral density function in the dipolar expressions for T_1, T_2, and NOE, Henry et al. (1986) analyzed ^{13}C-enriched alanine methyl group relaxation times for the coliphage M13 coat protein. They used a graphical approach to extract τ_m, S^2, and τ_e. The analysis required the direct measure of T_2 by spin echo rather than line widths, since the latter contained substantial contributions from chemical shift differences in some cases. Alternatively, if τ_m is known from other measurements, then S^2 and τ_e can be calculated easily by using only T_1 values at two different NMR frequencies or T_1 and NOE at a single frequency (Lipari & Szabo, 1982a).

With data at two different NMR frequencies, A and B, the order parameter and time constant are

$$S^2 = (R_A - R_B)/(R^0_A - R^0_B) \quad (1)$$

$$\tau_e = K(R_A R^0_B - R_B R^0_A)/(R_A - R^0_B - R^0_A + R_B) \quad (2)$$

where R_A and R_B are measured longitudinal relaxation rates, R^0_A and R^0_B are the calculated rates for a carbon with the same number of bonded protons undergoing isotropic reorientation with a correlation time of the whole macromolecule, and K contains constants of the dipolar interaction.

Alternatively, the order parameter can be calculated from T_1 and NOE data at a single frequency:

$$S^2 = \frac{T_1^0(2.988 - \mathrm{NOE})}{T_1(2.988 - \mathrm{NOE}^0)} \quad (3)$$

where T_1^0 and NOE^0 are the values expected with no internal motion at the NMR frequency of interest.

The order parameter for a methyl group contains a major contribution from motion about the axis. This motion is certainly fast and likely to be diffusional or to consist of random jumps between potential minima, in which case the measured generalized order parameter can be factored:

$$S^2 = S^2_{Rot} S^2_{ax} \quad (4)$$

Given the standard methyl group geometry, $S^2_{Rot} = 0.111$. Then

$$S^2_{ax} = S^2/0.111 \quad (5)$$

The term S^2_{ax} represents the order parameter for the methyl group axis. Most of the methyl groups in tRNA^{Phe} are attached directly to, or are one bond away from, a modified base ring or a ribose. Measuring the amplitude of motion of these

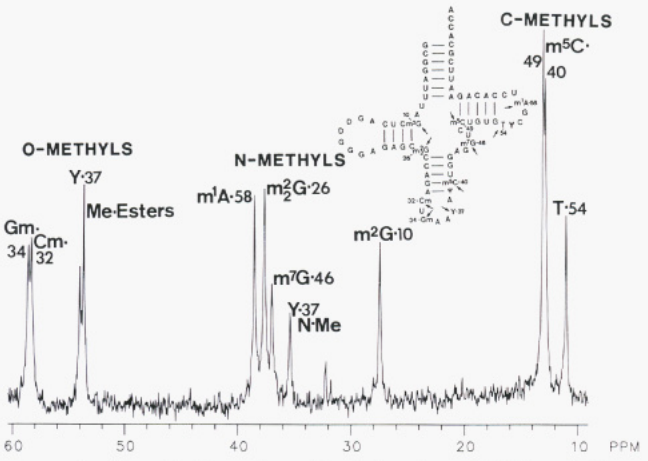

FIGURE 1: Cloverleaf two-dimensional structure of [^{13}C]methyl-enriched yeast tRNA^{Phe} with its carbon NMR spectrum. The specific sites of ^{13}C-methylation are indicated in the cloverleaf structure by arrows denoting the nucleotide position in the sequence. Each nucleotide has one ^{13}C-enriched methyl group except m_2^2G (two) and Y (three). The figure shows only the methyl region of the ^{13}C NMR spectrum and demonstrates the spectral separation of carbon-bound methyls, nitrogen-bound methyls, and oxygen-bound methyls.

groups relative to the overall molecule is the main thrust of the present study. Therefore, S^2_{ax} contains the desired information on spatial restriction of bases and ribose rings.

It is more difficult to extract analogous rates of motion from the correlation time, τ_e. The calculated value is dominated by rapid methyl group rotation around the axis, and there is no straightforward way to separate out that time dependence.

Results

Carbon-13 NMR spectra of yeast tRNA^{Phe} are shown in Figure 1. Peaks in the region 10–60 ppm are from methyl groups isotopically enriched in vivo by provision of [*methyl*-^{13}C]methionine to *Saccharomyces* cultures. A single peak at 120.9 ppm, also enriched from the labeled methionine, is assigned to ring carbon-10 in the nucleoside 3′ adjacent to the anticodon wyosine, Y-37 (Figure 2) (Agris et al., 1986). The methyl carbon resonances have been assigned by comparison of chemical shifts with a series of modified base nucleosides and by correlation of the proton and carbon chemical shifts in two-dimensional heteronuclear proton–carbon correlation spectroscopy (Agris et al., 1986). The 13 methyl groups in tRNA^{Phe} enriched in ^{13}C are described by 12 resolved individual peaks in the NMR spectrum. The two methyls of N^2,N^2-dimethylguanosine-26 (m_2^2G) produced a single, albeit broadened, peak.

Values of T_1 and NOE at 22.5, 75.5, and 118 MHz are listed in Table I. Line widths were measured for peaks in a spectrum at 75.5 MHz. They are also included in Table I, corrected for digital resolution and B_0 inhomogeneity. Several resonances show evidence of contributions from more than one chemical shift. The peaks for the Y-37 methyl ester at 54 ppm and both nucleosides containing ribose methylated at the 2′ position (Gm-34 and Cm-32) are distinctly nonsymmetric when examined closely. These nuclei appear to sample two or more local conformations that interconvert slowly relative to the chemical shift differences they produced. In any case, T_2 values for these peaks cannot be extracted from the line widths. Other tRNA^{Phe} peaks may also have (unresolved)

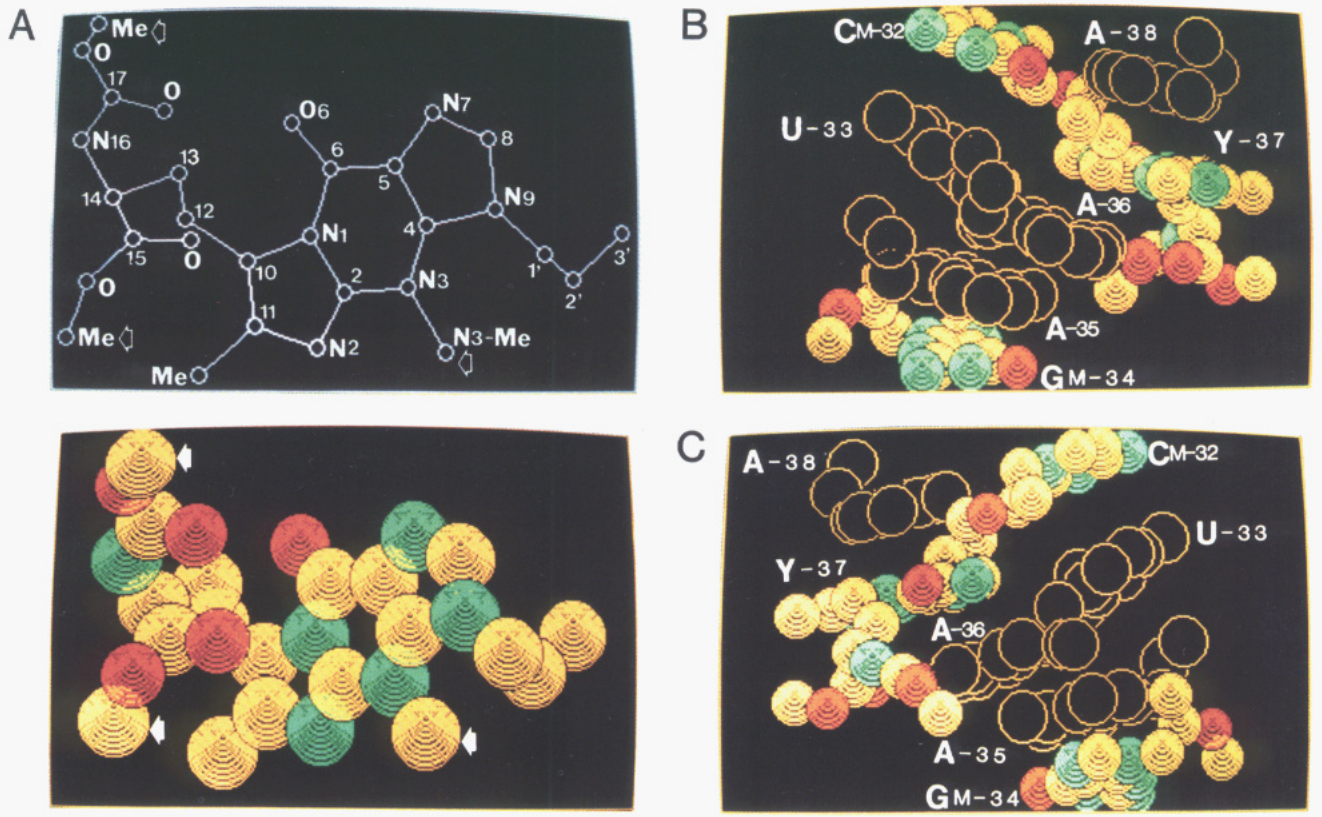

FIGURE 2: Structure and conformation of nucleoside Y and the anticodon of tRNA^{Phe}. The structure of nucleoside Y within yeast tRNA^{Phe} is presented in (A). The base and ribose carbons 1′, 2′, and 3′ are pictured. Hydrogens are not shown. Carbon-13 enriched methyl carbons are denoted by arrows. The nucleoside structures of the anticodon loop region with the Y nucleoside are presented in (B) and (C). Bases Cm-32, Gm-34, and Y-37 are pictured in full color, whereas U-33, A-35, A-36, and A-38 are shown with open circles. No hydrogen atoms or phosphates are shown. The base and ribose carbons 1′, 2′, and 3′ are depicted for each nucleoside. The codon-binding side is shown in (B), whereas the opposing view is shown in (C). Parts A–C were produced from the Cartesian coordinates of the respective X-ray crystallographic data by means of the space-filling, molecular graphics computer program, PC model version II (Academic). Carbon atoms are tan; nitrogens, blue-green; and oxygens, red.

Table I: Carbon Chemical Shifts, Line Widths, NOE Value, and T_1 Value for Three Different Field Strengths for Yeast tRNA^{Phe}

methyl group	chemical shifts (ppm) (TMS = 0 ppm)	line width (Hz) at 75.5 MHz	NOE[a] (1 + η) at 22.5 MHz	NOE[a] (1 + η) at 75.5 MHz	NOE[a] (1 + η) at 118 MHz	T_1 values[b] (s) at 22.5 MHz	T_1 values[b] (s) at 75.5 MHz	T_1 values[b] (s) at 118 MHz
T-54	11.0	12.5	1.72	1.85	2.07	0.374	1.75	3.98
m^{5}C-40, -49	12.9	17.0		2.20	2.46		0.99	1.04
			1.70			0.282		
m^{5}C-49, -40	13.0	11.5		2.20	2.70		1.09	1.36
m^{2}G-10	27.4	11.7	1.89	1.70	2.02	0.392	2.20	1.83
Y-37 *N*-methyl	35.4	12.0	1.78	1.40	2.25	0.236	1.56	1.90
m^{7}G-46	36.9	16.0	1.04	1.30	1.51	0.349	1.28	1.52
m$^2_2$G-26	37.6	17.0	1.40	1.40	1.36	0.362	1.88	2.00
m^{1}A-58	38.4	13.0	1.35	1.70	2.70	0.330	1.47	1.20
Y-37 methyl esters	53.6	14.0			1.85		0.88	0.90
			1.25	1.60		0.290		
	53.3	12.0			1.49		0.93	1.30
Cm-32	58.6	14.0						2.62
			1.15	1.60	1.50	0.296	1.28	
Gm-34	58.9	12.0						3.45
Y-37 C_{10}	120.7						3.80	

[a] Accuracy of NOE values: ±0.10. [b] Accuracy of T_1 values: ±0.05.

Table II: Order Parameters and Correlation Times for Internal Motion

nucleoside	group	S^2 A[a]	S^2 B[a]	S^2 C[a]	S^2 av ± SD	S^2_{ax}	τ_e[b] (ps)
m^{2}G-10	N-CH_3	0.074	0.059	0.053	0.062 ± 0.011	0.56	5.9
m$^2_2$G-26	N(CH_3)$_2$	0.062	0.062	0.060	0.061 ± 0.001	0.55	6.0
Cm-32	O-CH_3	0.087	0.073	0.079	0.080 ± 0.007	0.72	6.6
Gm-34	O-CH_3	0.087	0.073	0.082	0.081 ± 0.007	0.73	5.8
Y-37	N-CH_3	0.126	0.100	0.098	0.108 ± 0.016	0.97	5.7
Y-37	O-CH_3	0.073	0.064	0.062	0.066 ± 0.006	0.59	16.2
Y-37	O-CH_3	0.073	0.066	0.071	0.070 ± 0.003	0.63	12.7
m^{5}C-40[c]	C-CH_3	0.065	0.071	0.067	0.068 ± 0.003	0.61	13.7
m^{7}G-46	N-CH_3	0.075	0.058	0.058	0.063 ± 0.007	0.57	9.5
m^{5}C-49[c]	C-CH_3	0.065	0.073	0.074	0.070 ± 0.004	0.63	10.8
T-54	C-CH_3	0.048	0.059	0.064	0.057 ± 0.008	0.51	4.2
m^{1}A-58	N-CH_3	0.076	0.066	0.058	0.067 ± 0.009	0.60	10.0

[a] Method A used T_1 and NOE data at 22.5 MHz; B used T_1 data only at 22.5 and 75.5 MHz; C is for T_1 data at 22.5 and 118 MHz. [b] Average for eq 2 using 22.5–75.5- and 22.5–118-MHz T_1 data. [c] Assignments may be reversed.

contributions to line widths from chemical shift heterogeneities or exchange broadening; therefore, we did not use line width derived T_2 values in the following analysis.

From expressions for a generalized order parameter and time constant (eq 1 and 2, Experimental Procedures) the values of S^2 and τ_e were calculated, respectively. The value chosen for τ_m was 25 ns, equal to the result from fluorescence depolarization experiments on yeast tRNA^{Phe} (Tao et al., 1970) and close to the average of values obtained in other experiments on various fractionated and unfractionated tRNAs (Schmidt et al., 1980, 1983). More recently, studies of molecular motion of tRNA-size DNA that had been monitored by fluorescence depolarization reported the same range overall correlation times (Härd & Kearns, 1986).

With this τ_m value for the whole molecule, longitudinal relaxation rates for a methyl carbon, R^0_A and R^0_B (eq 1 and 2), were calculated on the basis of dipolar relaxation of a rigidly bound carbon due to three protons at 1.09-Å bond distance. The results for S^2 and τ_e were not particularly sensitive to choice of τ_m, varying about ±10% over the range 20–32 ns.

When data at 75.5 versus 118 MHz were used for calculating S^2 and τ_e with eq 1 and 2, the results in several cases were physically unreasonable (e.g., negative S^2 and τ_e). Some of this behavior is due to the fact that T_1 values at these frequencies are long and rather similar. Small experimental errors are magnified when relaxation rates at these two frequencies are subtracted. A more fundamental problem may be operative as well. This was illustrated when we calculated S^2 by eq 3 (Experimental Procedures), an alternative method to eq 1 and 2 that uses T_1 and NOE data at one frequency. Values of S^2 at 22.5 MHz (Table II) were all within 25% of those from the corresponding methyl group derived via eq 1. However, the same calculation (data not shown) for 75.5 MHz yielded values that averaged 2.4 times the data in Table II. Furthermore, with one exception (ribothymidine, T-54), all S^2 values were greater than 0.111, the maximum value of S^2 for a rotating methyl group. The model-free method appears to break down for the highest field data for tRNA. Lipari and Szabo (1982b) point out that inaccuracies result from the analysis if all internal motion is not in the extreme narrowing region. Methyl group reorientation on the axis is undoubtedly fast enough to be extreme narrowing ($\omega\tau_{int} \ll 1$). But it is likely that further motion of the methyl group axis is slow or intermediate on the NMR time scale, particularly at the higher fields used in this study (^{13}C frequencies of 75.5 and 118 MHz). For example, we have measured relaxation rates for ^{13}C-enriched ribose C1′ carbons of unfractionated tRNA and found an average of τ_{int} of 1.6 ns for the internal reorientation time of the C1–H vector (Schmidt et al., 1983). With other nucleic acids corresponding correlation times for sugar motions of about 0.5 ns have been measured (James & Schmidt, 1983). Spectral density terms in the ^{13}C–^{1}H dipolar interaction contain contributions at frequencies for ^{13}C and the sum and difference of ^{13}C and ^{1}H frequencies. If the relevant time scale is about 1 ns, then for the highest frequency, ^{1}H + ^{13}C, $\omega\tau_{int}$ = 0.71, 2.4, and 3.1 for 90, 300, and 470 MHz (^{1}H), respectively. The two higher NMR frequencies violate the

condition $\omega\tau_{int} < 1$ upon which the simple model-free analysis is predicted.

A further complication arises from possible chemical shift anisotropy (CSA) contributions to relaxation. Some indication that this mechanism is operative, at least at 75.5 MHz, comes from tRNA^{Phe} spectra taken with proton decoupling gated off during data acquisition. Unequal line widths are observed for individual transitions in the quartets. This behavior has been ascribed to the interference term between the dipolar and CSA relaxation mechanisms (Gueron et al., 1983; Anet, 1986; Farrar et al., 1986). The mathematical formalism of this phenomenon can serve as an additional means by which the motional parameters of tRNA may be calculated. The line broadening differential is not the same for every methyl group; the largest effect is about a ratio of 2 in line width for the two center transitions of Gm-34. In the face of complications with data at the higher magnetic field, we chose to average calculated S^2 values from analyses that were weighted toward the lowest field results, thus eq 1 was used with T_1 data at 22.5 vs 75.5 MHz or with T_1 data at 22.5 vs 118 MHz. Large values of relaxation rates ($1/T_1$) at 22.5 MHz dominate the result. Equation 3 employing T_1 and NOE at 22.5 MHz was also used for S^2 calculations. Averaging the three methods yielded values with a maximum standard deviation of ±18%.

From the average generalized order parameter, S^2, we calculated the order parameter for motion of the methyl group axis, S^2_{ax}, from eq 4. These values (Table II) directly relate to amplitudes of motion of the modified bases: Y-37 (N-CH_3), m^5C-40, m^7G-46, m^5C-49, T-54, and m^1A-58. Motion around the methylamine– and dimethylamine–ring bonds of m^2G-10 and m^2_2G-26 is highly restricted; therefore, the base motion is monitored by the axes of these methyls as well. For Cm-32 and Gm-34, S^2_{ax} measures possible motion of the C2′–O–C bond as well as ribose reorientation. Studies of unfractionated *E. coli* tRNA, enriched at C1′ with ^{13}C, yielded an average value of 18° for the half-angle of a cone in which the C1′–H vectors were constrained to move relative to the overall macromolecule (Schmidt et al., 1983). This semiangle corresponds to an order parameter of 0.89 (Lipari & Szabo, 1982b), compared to 0.72 and 0.73 for S^2_{ax} of Cm-32 and Gm-34 actually found in the present study. Full rotational mobility about the C2′–O bond would reduce the expected value to 0.014. The actual average values of 0.72 and 0.73 preclude free rotation and instead suggest very restricted mobility at these loci.

The Y-37 side chain is a unique structure terminated by two methyl esters (Figure 2). Order parameters of these methyls will be attenuated by motions of the rest of the links in the side chain and the wyosine base. Yet the values obtained, 0.59 and 0.63 for S^2_{ax}, are near those for methyls directly bonded to several other nucleosides in tRNA^{Phe}, suggesting that only very muted excursions occur for the Y-37 side chain atoms on a nanosecond or shorter time scale.

For those methyls whose axes follow the motion of a base, the S^2_{ax} values range from 0.51 to 0.97. The latter value, for Y-37, implies almost no motion relative to the whole macromolecule. Other S^2_{ax} values can be put into dimensional perspective if the motion is represented by diffusion within a cone. The semiangle ranges from 37° to 31°, which corresponds to S^2_{ax} values of 0.51–0.63. This is a rather limited range of amplitudes, suggesting that common forces are at work throughout tRNA to maintain structure.

Correlation times of internal motion, τ_e, range from 4 ps for T-54 to 16 ps for one of the Y-37 methyl esters. Rapid methyl rotations dominate, resulting in the small values observed. A survey of literature on protein and polymer NMR relaxation revealed a wide range of τ_e values for methyl groups calculated by the model-free method (Lipari & Szabo, 1982b; Henry et al., 1986). Our results fall into the range reported, generally at the small values end.

Discussion

Our model-free analysis of ^{13}C NMR relaxation rates and NOEs focuses on *amplitudes* of *fast* (subnanosecond) motion of selected bases and ribose rings relative to the overall structure. A surprising picture emerges from this work. Nucleotides with the most restricted internal motions are in the anticodon loop of tRNA^{Phe}.

The most restricted locus is the Y-37 base. Possibly because of its three-ring structure, this modified base must be well anchored by stacking interactions with adjacent rings. Even the Y-37 side chain is restricted in its mobility, suggesting specific interactions with nearby groups. Even though they are involved in tertiary hydrogen bonding, m^2G-10, m^2_2G-26, T-54, and m^1A-58 undergo the largest amplitude excursions that we measured.

In their analysis of the yeast tRNA^{Phe} crystal structure, Holbrook et al. (1978) examined the thermal parameters of bases, riboses, and phosphates. The largest temperature factors were found in the anticodon arm and loop and in the acceptor arm. Temperature factors are a measure of flexibility, and this result would seem to run counter to the NMR analysis reported here. However, as Holbrook et al. (1978) point out, the apparent thermal vibration measured from the crystallographic temperature factor includes "swaying, bending, or precessing motion of arms and also the partial opening and closing (unwinding and winding) of the double helical stems". These sorts of motion, which can involve large segments of the molecule, are likely to be on a time scale slower than nanoseconds and would not contribute to ^{13}C relaxation rates as measured here.

There is substantial evidence for conformational changes in the anticodon loop of tRNA^{Phe} and other tRNA in solution (Rigler & Wintermeyer, 1983; Bujalowski et al., 1986). Binding of Mg^{2+}, spermine, and oligonucleotides complementary to the anticodon sequence produces changes in this and other regions. In this regard the ^{13}C NMR spectrum of tRNA^{Phe} shows two peaks (in area ratios about 3 to 1) for each of the methyl resonances in the anticodon loop (H. Sierzputowska-Gracz, unpublished results). Chemical shift differences are small (less than 20 Hz at 75.5 MHz); the appearance of separate peaks implies slow exchange of the ^{13}C-labeled methyl groups between different magnetic environments. While there is apparently conformational lability of the anticodon loop as a whole, at least in the predominant form mobility of individual nucleosides is highly restricted.

Because methyl groups in tRNA^{Phe} produce separate, firmly assigned resonances, we are able to measure dynamics of individual nucleosides from locations throughout most of the macromolecule. This is the primary advantage of ^{13}C enrichment via [*methyl*-^{13}C]methionine. Although methyl group relaxation rates necessarily have a substantial contribution from (presumably) uninteresting rotation about the methyl axis, in the order parameter analysis that mode is easily factored out. We found, however, that the model-free analysis breaks down for high-field relaxation data, presumably due to the presence of internal motions that are not fast on the NMR time scale. We were successful in applying the model-free analysis because we had data from relatively low field (22.5 MHz). When only high-field relaxation data are available (e.g., because of signal-to-noise limitations), use of

the model-free analysis for tRNA and other nucleic acids may lead to inaccuracies.

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