^{1}H NMR studies of transfer RNA III: the observed and the computed spectra of the hydrogen-bonded NH resonances of baker's yeast transfer-RNAphe[1]

Lou S. Kan and Paul O. P. Ts'o

The Division of Biophysics, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205, USA

ABSTRACT

The hydrogen-bonded NH resonances of Baker's yeast tRNAphe in H_2O solution with Mg^{++} have been measured by a 360 MHz spectrometer at $23°C$. Totally, fifteen peaks and one shoulder can be resolved which represent 25 ± 1 protons. Based on the refined atomic coordinates of the tRNAphe in the orthorhombic crystal, on the recent advances in the distance dependence of the ring-current magnetic field effects[2] and on the adopted values for the isolated hydrogen-bonded NH resonances, a computed spectrum consisting of 23 protons was constructed. A quantitative comparison by computer was made between the computed spectrum and the spectrum simulated from the observed spectrum. These two spectra are closely similar but not identical. We suggest that the conformation of yeast tRNAphe in aqueous solution is closely similar but not identical to that found in the crystal, especially in the TψC region and D region. Also the NH resonances in 3-4 proposed hydrogen bonds (most likely for tertiary structure) may exchange very rapidly in aqueous solution.

INTRODUCTION

In the past two years, great advances have been made in the elucidation of the structure of yeast phenylalanine transfer ribonucleic acid in crystalline state by x-ray diffraction studies[3,4]. Recently the three dimensional coordinates of all the atoms (except hydrogens) in this tRNA molecule have been reported by four laboratories[5-8]. Although two crystal forms of the molecule have been examined by different groups, the structures determined from these two crystal forms are very similar to each other. Therefore, the next important question is: Does the common conformation of the tRNAphe determined in crystalline state also exist in aqueous solution? At present, nmr studies can provide a direct and defined answer to this question.

Following the early findings by Kearns, Shulman, and co-workers[9], the properties of the hydrogen-bonded NH-N proton resonances of yeast tRNAphe and of many other tRNA's have been extensively investigated with varying

degrees of sucess[10-14]. It should be noted that except in one or two iso-
lated cases[15,16], most of the NH-N resonances have not been unambigously
assigned. Calculations of chemical shifts of the NH-N resonances based on
the near-neighbour effect of ring-current magnetic anisotropy at a distance
of 3.4 Å[17] have provided encouraging results[14,18]. However, it should be
cautioned that such calculation is based on the assumption of the existence
of a clover-leaf conformation in solution, and ignores other magnetic fields
beyond or below a distance of 3.4 Å. Because of these assumptions and res-
trictions, the nmr results are not sufficiently conclusive or quantitative
to be compared directly with the results from x-ray diffraction studies in
detail.

In this communication, a calculation of the chemical shifts of the
NH-N proton resonance of yeast tRNAphe is presented. This calculation is
based on the three dimensional atomic coordinates of the crystalline tRNA
determined by x-ray diffraction studies and employs a graphic approach in
evaluating magnetic effects of the ring-current in a radius ranging from 5
to 10 Å and a vertical distance ranging from 3 to 8 Å from the bases. This
recently completed graphic approach eliminates the spatial restriction pre-
viously imposed on the ring-current calculation[2]. However, this calculation
still harbors the uncertainty about the adopted intrinsic values of the
chemical shifts of the isolated NH-N resonances, especially those from non-
Watson-Crick base pairs. In addition, when the assignment of all these
resonances in the experimental spectrum is not absolutely certain, the com-
parison of the calculated spectrum with the experimental spectrum is less
informative either in terms of understanding the conformation or validating
the calculation. However, valuable insight can still be gained in this
study, since the validity of this type of calculation has been shown in
several cases for the NH-N resonances in defined, short helical duplexes of
oligonucleotides[19-24]. Also, a similar approach has been reported in the
preceding papers[25,26] concerning the study on the methyl and methylene reso-
nances in the yeast tRNAphe originated from the minor nucleosides. In this
study, all the resonances have been assigned with certainty[25,26]. The
results on the NH-N resonances reported in this communication corroborate
with those on the methyl and methylene resonances. These findings suggest
the conformation of yeast tRNAphe in aqueous solution is grossly similar but
not totally identical to that in the crystalline state.

Recently, Robillard et. al.[27] reported a comparison between the cal-

culated and observed (360 MHz) ^{1}H nmr spectra of NH-N and NH-O resonances from yeast tRNAPhe. The observed spectrum (obtained at 35^0C with Mg^{++} as a personal communication from B. Reid) in this paper is nearly identical to ours. An integrated intensity of 26±1 protons was reported. Their calculations, on the other hand, are quite different from ours and the difference will be discussed in detail.

METHOD

Calculations of the chemical shifts of hydrogen-bonded NH resonances involve three major steps. Step (i): Determine the coordinates of the proton of interest and the spatial relation of this proton to its neighboring bases with a sphere of about 10 Å radius. In solving the former problem, the hydrogen-bonded proton is approximated to be in a line between two electronegative atoms (N or O) and the distance between this proton and the nitrogen atom (N_1 for guanine and N_3 for uracil or thymine) is 1.01 Å. The vertical (z) and radius (ρ) distances of this proton to its neighboring bases had been defined in the preceding paper[26]. Step (ii): Calculate the ring-current shielding (or deshielding) effect from the base to the proton from the vertical distance z and radial distance ρ. These shielding (or deshielding) effects in ppm can be directly obtained from the graphs of the ring-current versus z and ρ derived for the four different bases in paper of Giessner-Prettre, et. al.[2] Step (iii): Calculate the chemical shifts of these hydrogen-bonded proton resonances. Two parameters are needed in this step: First, the shielding (or deshielding) effect on the resonance (as described in Step ii); and second, the intrinsic chemical shift of the resonance of this proton in different base pairs. For the NH-N resonance in base pairs of Watson-Crick type, we adopted the values of Kearns and Shulman[11], i.e., 14.7, 13.4, and 13.6 ppm for AU, Aψ, and GC base pairs respectively. As for the tertiary hydrogen bonds, there are many types, most of which are not Watson-Crick base pair such as $G_{15}C_{48}$(Table I-B). Recently, Kallenbach, et. al.[21] have examined the $(U)N_3H-(A)N_7$ hydrogen-bonded proton resonance of reverse Hoogsteen type in an U·A·U triple stranded helix by ^{1}H nmr and recommended an intrinsic chemical shift of 14.1 ppm. From their data, together with a careful evaluation of the geometry and shielding effect, we estimated a value of 14.3 ppm as the intrinsic chemical shift of a reverse Hoogsteen $(U)N_3H-(A)N_7$ hydrogen-bonded proton resonance in an A'-RNA conformation[28]. As for the resonances of hydrogen-bonded NH-O in G·U, G·ψ, or G·C base pairs, as well as that of NH-N in G·A base pair, no intrinsic values of their chemical shifts have been determined experimental-

ly. Therefore, no calculations of the resonances of these five base pairs were made (Table I-B).

The atomic coordinates of the yeast tRNAphe in the orthorhombic crystal adopted in this calculation were kindly provided by Professor A. Rich of M.I.T. (A. Rich, private communication). The standard of deviation of this set of refined coordinates is approximately 0.2 Å. Preliminary trials indicate that computation based on the refined coordinates from A. Rich provides a better agreement with the experimental data than those based on the less refined coordinates[5-8].

The ^{1}H nmr spectrum of NH-N (or O) hydrogen-bonded resonances region was taken by a Bruker HX-360 MHz nmr spectrometer in Spectrospin AG, Fallanden-Zurich, Switzerland and with technical help from Mr. W. Schittenhelm. The sample solution contained 25 mg/ml purified yeast tRNAphe (kindly provided by Professor F. Cramer, Max Planck Institute, Gottingen, Germany), 0.01 M MgCl$_2$, 0.15 M NaCl, 0.002 M EDTA, and 0.01 M potassium phosphate buffer, pH=7. Totally, 1972 scans with 25 seconds sweep rate were accumulated for the spectrum in Figure 1.

The simulation spectra were done by a PDP-10 computer with a Calcomp 565 plotter at the medical computer center, University of Pennsylvania, Philadelphia, Pennsylvania. A "NLPLOT" simulation program was kindly provided by Dr. G. G. McDonald, also from the University of Pennsylvania.

RESULTS AND DISCUSSION

(A) The experimental spectra of yeast tRNAphe in the native state and the construction of an idealized experimental spectrum

Figure 1 shows the 360 MHz spectrum of the ^{1}H nmr resonances of the hydrogen-bonded NH from yeast tRNAphe sample at 23°C. Under this condition, the spectrum is essentially insensitive to temperature variation within ±10°C and can be considered as a reliable representation of the hydrogen-bonded NH resonances of yeast tRNAphe in native conformation. This spectrum has a good signal-to-noise ratio, and contains 15 well resolved peaks plus a shoulder (k') in the region of 11 to 15 ppm from DSS. This observed spectrum closely resembles the published and unpublished spectra of the same tRNA obtained under slightly different conditions[11,27](B. Reid, private communication).

A major problem in the quantitative analysis of these spectra concerns the exact number of protons represented in the spectra. One approach to this problem was to utilize the methyl resonance in the high field region (1 to 4 ppm) as the internal standard for the calibration[13], since it
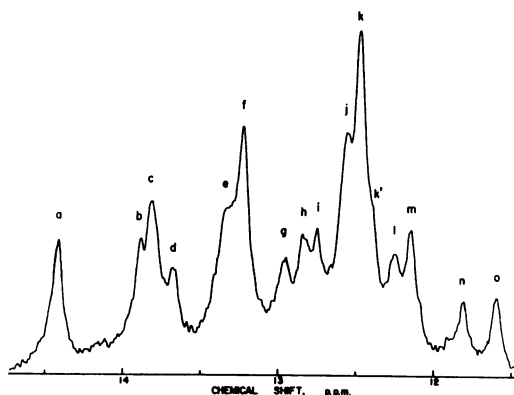
Figure 1. A 360 MHz spectrum of the yeast tRNA^{phe} at the 11.5-14.5 ppm region from DSS showing the hydrogen-bonded NH resonances at 23°C. The sample contained 25 mg/ml of tRNAphe, dissolved in 0.01 M MgCl$_2$, 0.15 M NaCl, 0.002 M EDTA and 0.01 M potassium phosphate buffer, pH 7.0.

has been found that the external standard[9] may not be satisfactory. While some success was claimed for E.coli. tRNA^{val}[13], this problem is more complex for yeast tRNAphe. If the methyl resonances are being used as internal standard, then they should be measured at the same temperature (23°C) as the NH resonances. However, at low temperature, these methyl resonances become broad and ill-resolved[25,26].

Bolton, et. al.[29] recently recalibrated the area under the hydrogen-bonded NH resonances in tRNA's by comparing them to the base proton resonances region of the same molecule in D$_2$O. They concluded that the yeast tRNAphe molecule has 22±1 NH protons in the -11 to -15 ppm region. This new number differs from the results of Reid and Robillard [13] and Robillard, et. al.[27]. The disagreement can be due to an assumption made by Bolton, et.al. [29] that no base protons have been shifted to other regions (for instance, the H$_6$ resonance of ψ which is located at -7.7 ppm and is shifted to -6.7 ppm in a dodecamer containing the anticodon loop[30]). Also, integration involving such large spectral area inevitably encounters the problem of base line[29]. Based on our own results on the integration of the NH resonances and the methyl resonances, we feel that it is safe to assume that isolated peak o represents one proton. With this assumed standard, the integration led to the following results: Peak a contains 2.0 protons; peaks b, c, and d contain 4.0 protons; peaks e and f contain 5.3 protons; peaks g, h, and i contain 2.6 protons, peaks j and k contain 6.8 protons, peaks l and m contain 1.9 protons; and peak n contains 0.8 protons. Totally 25±1 protons were obtained from the spectrum in Figure 1. Most of the uncer-

tainties come from the evaluation of the <u>baseline</u> of these peaks. This un-
certainty concerning the baseline would remain in spite of any solution to
the question of internal or external standard of integration. This uncer-
tainty of the baseline also creates problems for a direct and quantitative
comparison between the computed spectrum and the observed spectrum by a
computer procedure. For this reason, we decided to simulate the observed
spectrum by an idealized spectrum.

       Base on the experimental spectrum in Figure 1, the estimation that
the spectrum contains 25 NH's, a simulated spectrum was constructed
(Figure 2b). This simulated spectrum is considered to be the representa-
tive spectrum of the hydrogen-bonded NH resonances of yeast tRNA^{phe} in the
native state as observed in Figure 1. This simulated spectrum is used sub-
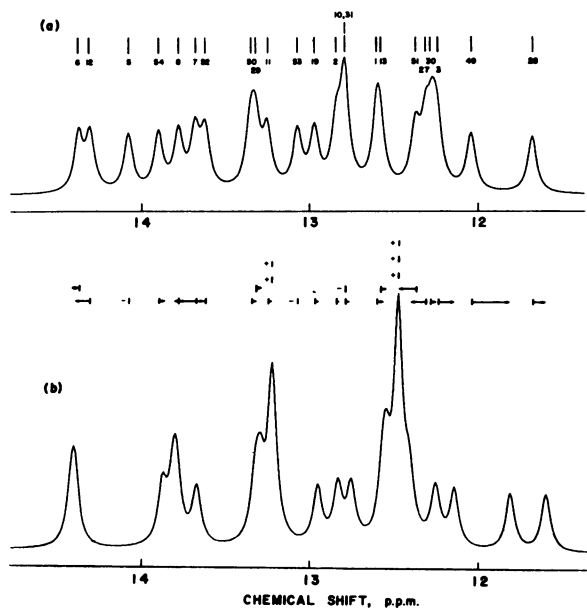

Figure 2.   (a) The computed spectrum of NH-N hydrogen-bonded proton resonances
in yeast tRNA^{phe} based on calculated chemical shifts shown in Table I.   The
number of these NH resonances represent the following base pairs:
6: U_6A_{67}, 12: $U_{12}A_{23}$, 5: A_5U_{68}, 54: $T_{54}m^1A_{58}$, 7: U_7A_{66}, 8: U_8A_{14}, 52: $U_{52}A_{62}$,
50: $U_{50}A_{64}$, 29: $A_{29}U_{41}$, 11: $C_{11}G_{24}$, 53: $G_{53}C_{61}$, 19: $G_{19}C_{56}$, 2: C_2G_{71},
10: $m^2G_{10}C_{25}$, 31: $A_{31}\psi_{39}$, 1: G_1C_{72}, 13: $C_{13}G_{22}$, 51: $G_{51}C_{63}$, 27: $C_{27}G_{43}$,
30: $G_{30}m^5C_{40}$, 3: G_3C_{70}, 49: $m^5C_{49}G_{65}$, and 28: $C_{28}G_{42}$.   Every computed peak
has an equal linewidth at half-height; 36 Hz in 360 MHz scale.
           (b) A simulated spectrum for the observed pscectrum shown in
Figure 1.   Every peak has a 36 Hz linewidth at half-height in 360 MHz scale.
The symbols between (a) and (b) represent the adjustments needed to trans-
form the computed spectrum (a) to the simulated spectrum (b); (-) represents
removal of resonance peaks, (+) represents addition of resonance peaks, and
(→ or ←) represent moving the chemical shifts to high field or low field,
respectively.

Table I. The Ring-current Magnetic Effect and the Calculated Chemical Shifts of the Hydrogen-bonded NH-N(O) Proton Resonances in a Yeast tRNA^{phe} Molecule.

A. Watson-Crick Type Base Pair

Base Pairs	Shielded By	5-Membered Ring	6-Membered Ring	Sum	Calculated Chemical Shifts[d]
G_1-C_{72}	C_2		0.04		
	G_3[a]	--	0.03		
	G_{71}	--	0.06		
	A_{73}	0.49	0.35		
	C_{74}[a]		0.03	1.00	12.60
C_2-G_{71}	G_1		0.07		
	G_3	0.12	0.24		
	C_{72}		0.14		
	A_{73}[a]	0.08	0.09	0.74	12.84
G_3-C_{70}	G_4	0.25	0.12		
	A_6[a]	0.06	0.07		
	G_{71}	0.27	0.54		
	C_{72}[a]		0.05	1.36	12.24
A_5-U_{68}	U_6		0.02		
	U_7[a]		0.01		
	A_{67}	--	0.43		
	U_{69}		0.13		
	C_{70}[a]		0.03	0.62	14.08
U_6-A_{67}	A_5	--	0.21		
	U_7		0.05		
	m^5C_{49}[b]		0.02		
	U_{68}		0.02		
	U_{69}[a]		0.02	0.32	14.38
U_7-A_{66}	m^5C_{49}		0.30		
	A_{67}	0.08	0.63		
	U_{68}		0.01	1.02	13.68
m^2G_{10}-C_{25}	A_9	0.06	0.04		
	C_{11}		0.11		
	U_{12}[a]		0.01		
	A_{23}[a]	--	0.05		
	G_{24}	--	0.05		
	m^2G_{26}	0.34	0.13		
	C_{27}[a]		0.02	0.81	12.79
C_{11}-G_{24}	m^2G_{10}	--	0.02		
	U_{12}		0.08		
	C_{13}[a]		0.05		
	A_{23}		0.08		
	C_{25}		0.04		
	$m_2^? G_{26}$	0.06	0.02	0.35	13.25
U_{12}-A_{23}	U_8[b]		0.01		
	C_{13}		0.25		
	A_{14}[a]	0.04	--		
	G_{24}	--	0.06		
	C_{24}		0.03	0.40	14.31
C_{13}-G_{22}	A_{14}	0.15	0.10		
	G_{15}[a]	0.05	0.04		
	A_{23}	0.17	0.54		
	G_{24}[a]	--	0.02	1.02	12.58
C_{27}-G_{43}	G_{26}	--	0.04		
	C_{28}		0.25		
	A_{29}[a]	0.05	0.06		
	A_{44}	0.12	0.68		
	C_{45}[a]	0.05	0.04	1.29	12.31
C_{28}-G_{42}	A_{29}	0.48	0.67		
	G_{30}[a]	0.06	0.02		
	G_{43}	0.17	0.35		
	A_{44}[a]	0.06	0.11	1.92	11.68
A_{29}-U_{41}	G_{30}	0.19	0.45		
	A_{31}[a]	0.09	0.12		
	A_{42}	0.35	0.11		
	G_{43}[a]	0.05	0.02	1.38	13.32
G_{30}-m^5C_{40}	A_{31}	0.19	0.96		
	C_{32}[a]		0.04		
	U_{41}		0.10		
	G_{42}	0.04	--	1.33	12.27

Base Pairs	Shielded By	5-Membered Ring	6-Membered Ring	Sum	Calculated Chemical Shifts
A_{31}-ψ_{39}	A_{38}	0.04	0.29		
	m^5C_{40}		0.27		
	U_{41}		0.01	0.61	12.79
m^5C_{49}-G_{65}	U_{50}		0.09		
	G_{57}	0.03	--		
	A_{66}	0.24	1.02		
	A_{67}[a]	0.07	0.11	1.56	12.04
U_{50}-A_{64}	G_{51}	0.44	0.15		
	U_{52}		0.01		
	G_{65}	0.25	0.31		
	A_{66}[a]	0.09	0.10	1.35	13.35
G_{51}-C_{63}	U_{52}		0.03		
	G_{53}[a]	0.08	0.04		
	A_{62}	--	0.12		
	A_{64}	0.28	0.64		
	G_{65}[a]	0.04	--	1.23	12.37
U_{52}-A_{62}	G_{51}	0.02	0.15		
	G_{53}	0.55	0.15		
	T_{54}[a]		0.01		
	C_{63}		0.01		
	A_{64}[a]	0.06	0.13	1.08	13.62
G_{53}-C_{61}	T_{54}		0.05		
	A_{58}[b]	0.07	0.01		
	A_{62}	0.16	0.22		
	C_{63}[a]		0.02	0.53	13.07
G_{19}-C_{56}	G_{18}	0.04	0.04		
	G_{57}	0.27	0.28	0.64	12.97

B. Non-Watson-Crick Type Base Pair

Base Pairs	Shielded By	5-Membered Ring	6-Membered Ring	Sum	Calculated Chemical Shifts
$G(H_1H)_4$-$U(O_2)_{69}$	A_5	0.18	1.12		
	U_6[a]		0.02		
	C_{70}		0.33		
	G_{71}	0.05	--	1.70	
$G(O_6)_4$-$U(H_3H)_{69}$	A_5	0.07	0.36		
	U_6[a]		0.01		
	C_{70}		0.15	0.59	
$U(H_3H)_8$-$A(H_7)_{14}$	C_{13}		0.17		
	G_{15}	0.08	0.05		
	G_{22}[b]	--	0.06		
	G_{23}[b]	0.04	0.12	0.52	13.78
$G(H_1H)_{15}$-$C(O_2)_{48}$	U_8[b]		0.01		
	G_{22}[b]	0.05	0.03		
	U_{59}[b]		0.02	0.11	
$G(H_1H)_{18}$-$\psi(O_4)_{55}$	G_{19}	--	0.03		
	C_{56}		0.04		
	G_{57}[a]	0.25	0.06		
	A_{58}[b]	0.35	0.40		
	C_{61}		0.04	1.17	
$G(H_7)_{22}$-$m^7G(H_1H)_{46}$	A_9[b]	0.21	0.58		
	A_{21}	0.15	0.76		
	G_{45}	--	0.03		
	C_{48}[a]		0.03	1.76	
$m_2^2G(H_1H)_{26}$-$A(H_1)_{44}$	A_9[b]	--	0.07		
	C_{27}		0.15		
	C_{28}[a]		0.03		
	G_{45}	0.06	0.10	0.41	
$T(H_3H)_{54}$-$A(H_7)_{58}$	G_{53}	--	0.09		
	$\psi 55$[c]		0.05		
	G_{57}	0.04	--		
	C_{61}[b]		0.09		
	C_{62}[b]	0.05	0.08	0.40	13.9

a   the next-nearest neighbor
b   through tertiary structure
c   the $\Delta\delta$ vs. z and r graph of U is used.
d   The adopted intrinsic chemical shifts are 14.7 for AU, 13.6 for GC, 13.4 for Aψ (all Watson Crick type) and 14.3 for AU. (reverse Hoogsteen type).

sequently in a comparison with the computed spectrum (Figure 2, Table II).

(B)   Computation of the chemical shifts of hydrogen-bonded NH resonances and the construction of the computed spectrum for the yeast tRNA[Phe] in native state

Table II. Comparison of the Computed Spectrum, Simulated
Spectrum and the Observed Spectrum of the Hydrogen-Bonded
NH Resonances of Yeast tRNA[Phe]

Computed Spectrum		Simulated Spectrum		Observed Spectrum		
base[a] pairs	chemical shifts(ppm)	chemical shifts(ppm)	adjustment[b] needed for transformation of the computed spectra to the simulated spectra (ppm)	chemical shifts(ppm)	peak[c] designation	approx. proton no.
6	14.38	14.42	0.04	14.41	a	2
12	14.31	14.40	0.09			
5	14.08	excluded		not observed		
54	13.90	13.875	-0.025	13.87	b	1
8	13.78	13.81	0.03	13.81	c	2
7	13.68		0.11			
52	13.62	13.67	0.05	13.67	d	1
50	13.35	13.32	-0.03	13.32	e	2
29	13.32	13.29	-0.03			
11	13.25	13.22	-0.03 (added 2 protons)	13.22	f	3
53	13.07	shifted		not observed		
19	12.97	12.95	-0.02	12.95	g	1
2	12.84	12.83	-0.01	12.83	h	1
31	12.79	12.75	-0.04	12.75	i	1
10	12.79	shifted		only 1 proton was observed in this region		
1	12.60	12.56	-0.04	12.55	j	2
13	12.58	12.54	-0.04			
51	12.37	12.47	0.10 (added 3 protons)	12.47	k	4
27	12.31	12.405	0.095	12.40	k'	1
30	12.27	12.25	-0.02	12.24	l	1
3	12.24	12.14	-0.10	12.14	m	1
49	12.04	11.81	-0.23	11.81	n	1
28	11.68	11.60	-0.08	11.60	o	1

(a) The designation number of the base pairs are described in the legend
     for Fig. 2a.
(b) Adjustments are shown in Fig. 2b.
(c) These peaks are shown in Fig. 1.

Table I shows the interatomic/intermolecular magnetic field experienced by the NH-N resonances in yeast tRNA[Phe] as computed by the ring-current effect based on the coordinates derived from the x-ray diffraction data. The sources of these magnetic field effects are listed and summed, which include next to the nearest neighboring bases or other nearby bases. As mentioned earlier, five putative hydrogen-bonded resonances from the non-Watson-Crick base pairs are not included in the calculation.

The data in Table I was plotted by the PDP-10 computer as shown in Figure 2a. As discussed above, this computed spectrum contains 23 resonances, six resonances less than the total recommended by the three dimensional structure of tRNA determined by x-ray diffraction[4,31].

(C) Quantitative comparison between the simulated experimental spectrum and the computed spectrum

The computed spectrum and simulated experimental spectrum are both displayed in Figures 2a and 2b. In addition, the adjustments needed to transform the computed spectrum to the simulated experimental spectrum are described in Table II and between Figures 2a and 2b.

Four general conclusions can be drawn from the above comparison. First, the simulated experimental spectrum and the adjustment of the computed spectrum to the simulated,experimental spectrum suggest that there are 25 ($\pm$1) NH resonances in the spectrum. This number of hydrogen-bonded NH (to N or O) is considerably larger than needed in the clover-leaf structure (20) but is also definitely smaller than that exhibited by the structure in crystal (29). The data suggest that 4 (or 3-5) NH's in the base pairs existing in the crystal structure may exchange with water rapidly so that they cannot be detected by nmr technique.

Second, within the tolerance range of $\pm$0.1 ppm, only four resonances out of 25 are shown to be incorrectly calculated by the ring-current procedure (Table II). This result not only suggests that the ring-current procedure is indeed useful, but also the intrinsic chemical shifts adopted are probably correct. In addition, the atomic coordinates adopted for the tRNA in solution may also be accurate for the most part, a conclusion which will be discussed further. Four computed resonances require more substantial adjustments. The first resonance is from the $m^5C_{49}G_{65}$ pair which gives the only computed peak within a close range to the observed peak at 11.81 ppm. Therefore, the NH resonance of this $m^5C_{49}G_{65}$ pair is tentatively assigned to the n peak at 11.81 ppm, though an adjustment of -0.23 ppm is needed. The second resonance is from the A_5U_{68} pair which is calculated to be at 14.08 ppm and is not within $\pm$0.1 ppm from any observed peak. This A_5U_{68} pair is immediately below the putative G_4U_{69} pair recommended in the crystal structure. Our current explanation is that this NH resonance is likely to be shifted upfield or this NH may exchange rapidly in water because its adjacent putative G_4U_{69} base pair may not survive in water[11,18]. The third resonance is from the $G_{53}C_{61}$ pair which is calculated to be at 13.07 ppm, and cannot be fitted to the observed spectrum by adjustment

within ±0.1 ppm. This base pair is located at the junction between the TψC loop and TψC stem. While this base pair most likely does exist, the NH-N resonance could be shifted elsewhere, such as to the f peak located 0.15 ppm downfield. The fourth resonance is either from $m^2G_{10}C_{25}$ pair or $A_{31}\psi_{39}$ pair; both are calculated to be at 12.79 ppm. Since only one hydrogen-bonded NH resonance was observed at 12.75 ppm, furthermore the second one cannot be accommodated to this spectral position within the range of ±0.1 ppm, one of these two NH resonances has to be removed or shifted elsewhere. The NH resonance in the $m^2G_{10}C_{25}$ pair, which is located at the beginning of D stem and at the center of the folded region, is the likely candidate for removal or shifting. Since the number of the observed hydrogen-bonded NH resonances (25±1) is less than the number suggested from the tRNA structure in the crystal, 3-4 proposed hydrogen-bonded NH's may exchange very rapidly in aqueous solution. At present, no definite infor-mation is available indicating which base pair recommended in the crystal structure may not be detectable by nmr in aqueous solution, though the G_4U_{69} is a good prospect. Also, the spectral location of the NH resonance of the putative G·U base pair cannot yet be calculated since no information is yet available about the intrinsic chemical shift of this NH-O resonance.

Third, while the comparison between the calculated spectrum and the simulated experimental spectrum may not depend on the assignment of all the NH resonances, certain definitive information about the assignments can be derived from this investigation. For instance, due to the isolated posi-tion of these resonances in both the experimental spectrum and the calcu-lated spectrum, as well as the close agreement between these two spectra about these resonances, the assignments of the NH resonances in U_6A_{67}, $U_{12}A_{23}$, and $C_{28}G_{42}$ are likely to be correct. In this case, the resonance observed at 11.6 ppm, now assigned to $C_{28}G_{42}$, is therefore unlikely to come from $C_{13}G_{22}$ (now assigned to 12.6 ppm) as recommended by Kearns and Shulman[11] . It is interesting to note that the NH resonance from the tertiary $G_{19}C_{56}$ Watson-Crick base pair[31] was calculated to be located at 12.97 ppm. Recently, the NH resonance of a tertiary hydrogen bond of $G_{15}C_{49}$ reverse Watson-Crick base pair in E.coli tRNA^{met}fl has been unambiguously assigned by Daniel and Cohn[15,32] at this chemical shift.

(D) Comparison among all the assignments and calculations on hydrogen-
bonded resonances.

Recently, Robillard et.al.[27] reported a calculated NH spectrum of yeast tRNA^{phe} to be compared with the 360 MHz nmr spectra obtained by B. R. Reid

under various experimental conditions. In their report, however, the sources for the new ring-current values contributed by individual bases, as well as the numbers employed for the actual calculations, were not described in detail. In addition, the calculations on the distance dependence of the ring-current effects were not presented. Nevertheless, the following discussion will still be informative based on the presented information. Robillard et.al.[27] have made all assignments based on their own calculation and estimation (see their Table III; including one typographical error on $A_{31}\psi_{39}$) and stated that the assigned values of these resonances are very close (within 0.1 ppm) to the observed values. Kearns and coworkers [10,11,14,18] have also made all the assignments based on their fragment data and their calculation. Kearns et.al. also reported that most of their assignments agree with the observed values within 0.1 ppm (except two out of twenty which are within 0.2 ppm). A careful comparison reveals that the differences between these two sets of assignments are generally in the range of 0.06 to 0.3 ppm, but four of them differ more than 0.5 ppm (Table III).

Table III. A comparison among the assignments by Robillard et al. (27), Kearns et al. (14) and the computed resonances presented in this communication.

Base Pairs	(a) Robillard et al.	(b) Kearns et al.	(c) This Communication	Differences Between		
				(a) and (b)	(a) and (c)	(b) and (c)
$G_1 C_{72}$	12.31	12.4	12.6	0.09	0.29*	0.20
$C_2 G_{71}$	12.47	12.7	12.84	0.23	0.37*	0.14
$G_3 C_{70}$	12.13	12.2	12.24	0.07	0.11	0.04
$A_5 U_{68}$	13.74	--	14.08	--	0.34*	--
$U_6 A_{67}$	13.74	14.3	14.38	0.56*	0.64*	0.08
$U_7 A_{66}$	13.31	13.2	13.68	-0.11	0.37*	0.48*
$G_{10}C_{25}$	12.55	12.7	12.79	0.25	0.24	0.09
$C_{11}G_{24}$	13.10	13.3	13.25	0.20	0.15	-0.05
$U_{12}A_{23}$	14.01	13.7	14.31	-0.31	0.31*	0.61*
$C_{13}G_{22}$	12.98	11.5	12.58	-1.48*	-0.40*	1.08*
$C_{27}G_{43}$	12.38	12.3	12.31	-0.08	-0.07	-0.22
$C_{28}G_{42}$	11.58	11.9	11.68	0.32*	0.10	0.01
$A_{29}U_{41}$	13.20	13.3	13.32	0.10	0.12	0.02
$G_{30}C_{40}$	12.60	12.4	12.27	-0.2	-0.33*	-0.13
$A_{31}\psi_{39}$	13.18	13.0	12.79	-0.18	-0.39*	-0.21
$C_{49}G_{65}$	12.19	12.4	12.04	0.21	-0.15	-0.36*
$U_{50}A_{64}$	12.81	13.3	13.35	0.49*	0.54*	0.05
$G_{51}C_{63}$	12.26	12.5	12.37	0.24	0.11	-0.13
$U_{52}A_{62}$	13.22	13.8	13.62	0.58*	0.40*	-0.18
$G_{53}C_{61}$	12.55	12.5	13.07	-0.05	0.52*	0.57*
$G_{19}C_{56}$	12.68	--	12.97	--	0.29	--
$U_8 A_{14}$	14.42	--	13.78	--	-0.64*	--
$T_{54}A_{58}$	14.38	--	13.9	--	-0.48*	--

*denotes a difference of larger than 0.3 ppm.

Robillard et.al.[27] have stated that "The assigned analysis of the yeast tRNA^{phe} nmr spectrum was made with considerable help from NMR spectra of fragments of the individual helices. .....it is immediately evident that the present assignments are in close agreement with the fragment data". We have compared the assignments by Robillard et.al.[27] and the fragment data

quoted by Robillard et.al.[27]   As shown in Table IV, definite disagreement appears to outweigh possible agreements between these two sets of values.

Table IV. A Comparison between the Assignments by Robillard, et. al. (27) and the resonance data from fragments (18).

Fragments	Base Pairs	Assignments by Robillard, et. al.	Resonance positions in fragments	Comments
21-57	$A_{29}U_{41}$	13.22	13.25	three possible
	$A_{31}\psi_{39}$	13.18	12.7	agreements with
	$G_{30}C_{40}$	12.57	12.5($\sim$two pro-	two definite
	$C_{27}G_{43}$	12.42	12.1 tons)	disagreements.
	$C_{28}G_{42}$	11.58		
34-76	$U_{52}A_{62}$	13.25	13.7	two possible
	$U_{50}A_{64}$	12.83	13.3	agreements with
	$G_{53}C_{61}$	12.49	12.7	three definite
	$G_{51}C_{63}$	12.27	12.46 ($\sim$three protons)	disagreements
	$C_{49}G_{65}$	12.23		
1-33	$U_{12}A_{23}$	13.91	14.2	one possible
	$C_{11}G_{24}$	13.16	13.4	agreement with
	$G_{13}C_{22}$	12.93	12.86	three definite
	$G_{10}C_{25}$	12.51	(one less proton)	disagreements

In this paper, while we have made calculations on these hydrogen-bonded NH resonance, we have not made definite assignments of them. A comparison between the assignments by Robillard et.al.[27] and our work is shown in Table III. Differences larger than 0.3 ppm are found in 13 sets of values. In addition, Robillard et.al.[27] have already mentioned the differences in their calculated values to the assignments by Kallenbach et.al.[21] in the study of AMP-oligo U triplex and to our experimental observation on $(A_2GCU_2)_2$ helix[19].

A comparison between the assignments made by Kearns et.al.[11],[14],[18] and this work is also shown in Table III. In general, these two sets of values are comparable, except for those base pairs (U_7A_{66}, $A_{31}\psi_{39}$, $G_{53}C_{61}$, and $C_{13}G_{22}$) located at the end of the short helices and for $U_{12}A_{23}$.

In summary, the sources of differences in the calculations among these three groups can be described as follows:

(i) The differences between the calculations by   Kearns et al.[11,14] and   this work are due to two major sources: (a) Only a clover-leaf model and the geometry of short helices were used in the calculation by Kearns et.al.[11],[14], whereas a three-dimensional model base on tRNA conformation in crystalline state was used in our work; (b) only the ring-current effects of the nearest neighbors in the short helices at a 3.4 $\overset{o}{A}$ distance were included by Kearns et.al.[11],[14], whereas the ring-current effects with a vertical distance of 3-8 $\overset{o}{A}$ and radial distance up to 10 $\overset{o}{A}$ were included by

us. However, both calculations employed the same intrinsic values for the isolated hydrogen-bonded NH resonances.

(ii) The differences in the calculations from Robillard et.al.[27] and from this work are due to the differences in the theoretical treatment of the ring-current effects and the hydrogen-bonded NH resonances, while both calculations were based on similar three-dimensional models of tRNA. Therefore, different intrinsic values for the isolated hydrogen-bonded NH resonances and different ring-current effects were employed in these two calculations.

This analysis clearly indicates that the current status of computation of nmr data may not be sufficiently certain for making definitive assignments of NH resonances in tRNA spectra. Nevertheless, valuable information can still be gained if the results are treated with proper caution.

(E) The conformation of native yeast tRNA[phe] in aqueous solution

As mention in the preceding paper[26], comparison between the x-ray diffraction and the nmr results can be meaningful only if the comparison is made on the basis of a three-dimensional model. Any disagreement in the comparison can be due to the difference in the conformational states (crystal vs. solution), or to inaccuracies in determination of conformation, or both. Despite some uncertainties in the theoretical treatment, this quantitative comparison between the simulated experimental spectrum and the calculated spectrum based on the atomic coordinates of the tRNA in crystal and on ring-current effects clearly indicates that the native conformation of yeast tRNA[phe] in solution is fundamentally similar to that in crystal state. The minor difference is probably in the tertiary structure involving the folding of the TψC loop and stem to the D loop and stem. This conclusion is reinforced by the ^{1}H nmr studies on the methyl/methylene resonances of the minor bases reported in the preceding paper[26]. In addition, some of the hydrogen-bonded base pairs existing in the tRNA in the cyrstal state may not be detectable in solution. The problem concerning the theoretical aspects of the ring-current effects and the intrinsic values of hydrogen-bonded NH resonance undoubtedly deserves further attention.

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1. This paper is dedicated to JEROME VINOGRAD.

   Abbreviations used: yeast tRNA^{phe}-Baker's yeast phenylalanine transfer ribonucleic acid; A-adenosine; C-cytidine; G-guanosine; U-uridine; ψ - pseudouridine; T-ribothymidine; m^{2}G-N^{2}methylguanosine; D-dihydrouridine; m$^2_2$G-N^{2,2}dimethylguanosine; m^{1}A-1-methyladenosine; m^{5}C- 5-methylcytidine; m^{7}G- 7-methylguanosine; nmr-nuclear magnetic resonance; ppm-parts per million; DSS-2,2-dimethyl-2-silapentane-5-sulfonate.

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