The crystal structure of yeast phenylalanine tRNA at 1.93 Å resolution: A classic structure revisited

HUIJING SHI and PETER B. MOORE

Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, USA

ABSTRACT

The crystal structure of the monoclinic form of yeast phenylalanine tRNA has been redetermined at a resolution of 1.93 Å. The structure of yeast tRNA^{Phe} described here is more accurate than its predecessors not only because it incorporates higher resolution data, but also because it has been refined using techniques that had not been developed when its predecessors were determined more than 20 years ago. The 1.93 Å resolution version of this structure differs interestingly from its predecessors in its details. In loop regions particularly, the backbone torsion angles in the new structure are not the same as those reported earlier. Several new divalent cation binding sites have been identified, and the water structure that has emerged is also different.

Keywords: crystal structure; phenylalanine; tRNA; yeast

INTRODUCTION

One of the most important achievements in structural biology in the 1970s was the determination of the crystal structure of yeast phenylalanine transfer RNA. The excitement generated in 1974 by the announcements from Klug's group in Cambridge and from Rich's group at the Massachusetts Institute of Technology (MIT) of their 3 Å resolution structures of the monoclinic (Robertus et al., 1974) and orthorhombic (Kim et al., 1974) crystal forms of yeast tRNA^{Phe}, respectively, remains etched in memory. Two years later, Sundaralingam and coworkers announced an independent determination of the structure of the monoclinic crystals of the same molecule, also at 3 Å resolution (Stout et al., 1976), and ultimately four sets of coordinates for yeast tRNA^{Phe} were deposited in the Protein Data Bank: (1) 4tna (Hingerty et al., 1978), the Cambridge monoclinic structure refined at 2.5 Å resolution, (2) 6tna (Sussman et al., 1978), the MIT orthorhombic structure, refined to 2.7 Å resolution, (3) 1tra (Westhof & Sundaralingam, 1986), Sundaralingam's monoclinic structure refined at 3.0 Å, and (4) 4tra (Westhof et al., 1988), a version of the MIT orthorhombic refined by a different method, also at 3.0 Å resolution.

These studies were significant not only because yeast tRNA^{Phe} was the first naturally occurring RNA to have

its crystal structure determined, but also because it was not until 1994 that a crystal structure appeared for another RNA that large that was not a tRNA (Pley et al., 1994). Thus for two decades these tRNA structures were scrutinized with unrelenting intensity, and much made of the details of their conformations, even those that cannot be determined unambiguously at 2.5–3.0 Å resolution.

Our interest in these structures resulted from a computational investigation of the feasibility of determining the solution structures of tRNA-sized RNAs by NMR, which used the crystal structure of yeast tRNA^{Phe} as its starting point. In the course of this work, it became clear that even though the backbone trajectories and base placements are similar in all of the versions of this structure deposited in the Protein Data Bank, they differ significantly in detail, particularly with respect to backbone torsion angles, to which NMR is quite sensitive (Table 1).

Because there have been significant advances in crystallographic techniques over the past quarter century, there is no reason why uncertainties about the conformation of this important molecule need persist. The structures mentioned above were completed at a time when macromolecular crystal structure refinement was in its infancy; none of them would be regarded as adequately refined today. In addition, the development of frozen crystal techniques, synchrotron light sources and high-sensitivity area detectors have revolutionized crystallographic data collection. Crystals that yielded 2.5 Å resolution data in the 1970s are all but guaranteed to

Reprint requests to: Peter B. Moore, Department of Chemistry, Yale University, 350 Edwards Street, New Haven, Connecticut 06520-8107, USA; e-mail: moore@proton.chem.yale.edu.

TABLE 1. Coordinate and torsion angle comparisons.

Rmsd torsion angle (°)	Rmsd (Å)			
	4tna	1tra	4tra	6tna
4tna		0.58	1.18	1.18
1tra	28.5		1.17	1.17
4tra	42.9	44.6		0.44
6tna	45.7	46.9	27.9	

produce significantly higher resolution data today. Thus it was clear from the outset that a more accurate understanding of the structure of yeast tRNA^{phe} would emerge if its crystal structure were revisited.

We report a study of the monoclinic crystals of yeast tRNA^{phe} that has resulted in a 1.93 Å resolution structure for that molecule. The overall architecture of the molecule apparent in the 1.93 Å resolution version of its structure is the same as that first described at lower resolution more than 25 years ago, but there are interesting differences in detail. First, the data indicate that residues D16 and U59 (see Fig. 1) form a base pair when Mn²⁺ or Co²⁺ is present in these crystals, but when Mg²⁺ is the only divalent cation present, D16 adapts the orientation reported earlier in orthorhombic crystals. Second, it is clear that the backbone torsion angles of nucleotides in loop regions in all of the existing structures need revision. Third, the number of sites where divalent metal ions bind in the monoclinic form of yeast phenylalanine tRNA appears to be 11, not 5, as originally reported.

RESULTS

Data quality and structure refinement

As the statistics in Table 2 demonstrate, data sets can be obtained from monoclinic crystals of yeast tRNA^{phe} today that do indeed extend to significantly higher resolutions than those collected in the 1970s. As it happened, the best data we obtained came from a crystal soaked in a Mn²⁺-containing buffer (see Materials and methods). Figure 2 shows the TΨC loop region of the electron-density map derived from that data set, and Table 2 provides statistics that characterize the structure obtained from it, as well as statistics for the structures obtained from the other two data sets summarized in Table 2.

A cross-validated Luzzati plot of R-factor against resolution (Luzzati, 1952) indicated that the expected coordinate error of the Mn²⁺ structure is about 0.32 Å (data not shown). This estimate is consistent with the root mean squared differences (rmsd) in non-hydrogen atom positions between this structure and the lower resolution structures obtained from the other two data sets, one of which was collected from crystals soaked in a Co²⁺-containing buffer (rmsd = 0.66 Å) and the other of which was collected from crystals that were not exposed to transition metal ions (rmsd = 0.48 Å).

Initial impressions of the high-resolution structure

Figure 3 shows the entire tRNA molecule in ribbon format as well as the positions of its associated divalent

TABLE 2. Data sets and refinement statistics.^a

	Mn ²⁺	Co ²⁺	Native
Data collection			
Wavelength (Å)	0.913	0.913	0.9806
Unique reflections (N)	17,365	15,002	12,032
Mean redundancy	13.7	11.6	7.9
Completeness (%)	89.9 (83.3)	92.2 (52.2)	98.5 (96.0)
R_{merge}^b (%)	3.9 (28.5)	4.8 (35.3)	7.9 (50.9)
I/σ	41.5 (3.6)	34.2 (2.1)	17.1 (2.1)
Space group	P2 ₁	P2 ₁	P2 ₁
a, b, c (Å)	54.9, 33.4, 61.9	54.3, 33.8, 61.9	55.3, 32.9, 62.2
β (°)	90.20	90.35	90.6
Refinement			
Resolution (Å)	40.0–1.93	40.0–2.0	40.0–2.2
Reflections			
working set (N)	15,371	12,862	9,849
test set (N)	1,525	1,458	1,127
R-factor ^c (%)	23.34	27.29	24.22
R-free ^c (%)	25.30	29.85	26.91
Rmsd bond length (Å)	0.012	0.0055	0.0051
Rmsd bond angle (°)	1.605	1.126	1.069

^aThe numbers in the parentheses are the values at the highest shells.

^b $R_{merge} = \sum |I_{obs} - \langle I \rangle| / \sum \langle I \rangle$, where I is diffraction intensity and $\langle I \rangle$ is mean measured intensity.

^cR-factor and R-free = $\sum ||F_{obs}| - |F_{calc}|| / \sum |F_{obs}|$, where R-free is computed using the 10% of the data.

FIGURE 1. A: The secondary structure of yeast phenylalanine transfer RNA. The U₆-A₆₇ base pair found in the acceptor stem in the more abundant of the two isoacceptors is replaced by C₆-G₆₇ in the less abundant form. **B:** The sequence of yeast phenylalanine transfer RNA. This molecule contains 11 modified bases. They are: 2-methyl-guanosine (2MG), 5,6-dihydrouridine (DHU), N²-dimethylguanosine (M2G), O²-methyl-cytidine (OMC), O²-methyl-guanosine (OMG), the Y base or wybutosine (YG), pseudouridine (PSU), 5-methyl-cytidine (5MC), 7-methyl-guanosine (7MG), 5-methyl-uridine (5MU), and 1-methyl-adenosine (1MA).

cations. The RNA is oriented with its anticodon loop pointed down, its TΨC loop facing the viewer at its elbow, and its acceptor stem pointed to the right. Not surprisingly, at this level of description, the 1.93 Å resolution version of this RNA is all but indistinguishable from any of its four lower resolution antecedents, except for its metal-binding sites, about which more will be said below. In fact, the position of only a single nucleotide in the entire molecule, D16, is called into question by the new data.

Figure 4 shows stereo views of the D-loop region of composite omit maps obtained for the Mg²⁺ structure (Fig. 4A), the Mn²⁺ structure (Fig. 4B), and the Co²⁺ structure (Fig. 4C). In each panel, our interpretation of that electron density is shown in cyan, the old monoclinic interpretation (4tna) is shown in magenta, and the old orthorhombic (4tra) interpretation is depicted in green. Clearly the position of D16 is sensitive to ionic conditions. When Mg²⁺ is the only divalent cation present, D16 points away from U59. In solvents that

FIGURE 2. The final, 1.93 Å resolution composite omit map in the TΨC loop (residue 53–61) region with the structural interpretation superimposed. The electron-density map shown is contoured at a level of 1.0 σ . The figure was prepared using SPOCK (Christopher, 1998).

include Mn^{2+} or Co^{2+} as well as Mg^{2+} , D16 points towards U59. In the Co^{2+} case, D16 and U59 form a base pair in which the O2s of both bases are hydrogen bonded to the imino protons of their partners (Saenger

type XIII) (Saenger, 1984). The placement of D16 observed in our native, monoclinic crystals is close to that previously reported for the orthorhombic crystal form of this molecule, and its placement in our Mn^{2+} and Co^{2+} structures resembles that reported previously for the monoclinic crystal form of the molecule.

FIGURE 3. The overall conformation of yeast phenylalanine tRNA and distribution of divalent cation-binding sites in tRNA^{phe} . The tRNA is shown as a ribbon diagram with the approximate orientations of its bases indicated. Metal-binding sites located in the original four structures are colored yellow. Newly identified Mg^{2+} -binding sites are shown in green. Sites specific for heavier metals are purple. This figure was prepared using RIBBONS (Carson, 1991).

Torsion angles

The torsion angles of the 1.93 Å resolution structure and their standard deviations are listed in Table 3. [For torsion angle definitions see Saenger (1984).] The standard deviation estimates given were computed using the values of corresponding torsion angles in the three tRNA structures described in Table 2, which are independently refined structures derived from data collected from different crystals. In this transfer RNA as in other RNAs, the torsion angles of the vast majority of nucleotides are those characteristic of A-form helix. Reading from α to ζ , their rotamer ranges are g $^-$, t, g $^+$, g $^+$, t, and g $^-$, and the glycosidic torsion angle is *anti*-. Even in the less regular regions of the molecule, nucleotide conformations tend to be A-form-like.

Divalent cation-binding sites

The positions of the 11 divalent cation-binding sites detected in the course of this study are indicated in Figure 3, colored to distinguish Mg^{2+} sites identified in earlier crystallographic studies (yellow) both from Mg^{2+} sites described here for the first time (green) and from sites to which only transition metal cations appear to bind (purple). Figure 5 shows the metal-associated elec-

FIGURE 4. The dependence of the structure of the D16 region on cation identity. **A:** Native, Mg^{2+} structure; **B:** the Mn^{2+} structure; **C:** the Co^{2+} structure. In each case our interpretation of the map is shown in cyan, and for reference the monoclinic structure (4tna) is shown in magenta, and the orthorhombic structure (4tra) in green. The maps in question are composite omit maps contoured at the 1 σ level.

tron density at all 11 sites as it appears in composite omit maps. In the case of sites M1–M7, the metal shown is Mg^{2+} . The metal in sites M8 and M9 is Mn^{2+} , and M10 and M11 are shown with Co^{2+} bound. The metal ions observed are octahedrally coordinated in all of these sites except M5, which is located in the anticodon loop (Fig. 5E), and the electron density associated with sites M7, M8 (Fig. 5C), and M6 (Fig. 5F) is relatively weak, which suggests that those sites may not be fully occupied under the conditions explored here.

Sites that bind Mn^{2+} and Co^{2+} preferentially were identified using $[(\text{Mn}^{2+} \text{ or } \text{Co}^{2+}) - \text{Mg}^{2+}]$ difference Fourier maps, and because the solvents containing

those two ions included Mg^{2+} at a concentration 20-fold higher, only sites that bind these heavier metal ions with an affinity considerably higher than Mg^{2+} could be detected. Mn^{2+} preferentially replaces the Mg^{2+} ions normally found in sites M2 and M8, and it binds at an additional site that Mg^{2+} does not appear to occupy, M9. Co^{2+} also displaces Mg^{2+} from sites M2 and M8, and it binds to M9 as well as two additional sites, M10 and M11.

Because there is a weak anomalous component to the scattering factors of both Mn and Co at the X-ray wavelength used here ($f'_{\text{Mn}} = 1.14$ electrons, $f'_{\text{Co}} = 1.56$ electrons), the Mn^{2+} and Co^{2+} data sets were used to compute anomalous difference electron-density maps

TABLE 3. Mn²⁺ torsion angles (°).

	Residue number	α	β	γ	δ	ϵ	ζ	χ
Acceptor stem	G1	0	0	66(131)	93(4)	203(9)	296(4)	189(3)
	C2	290(68)	187(4)	54(6)	82(1)	213(18)	285(10)	200(9)
	G3	297(13)	172(10)	58(3)	81(2)	211(8)	281(9)	199(5)
	G4	293(8)	162(9)	63(2)	83(1)	206(2)	290(1)	189(3)
	A5	289(3)	182(0)	51(3)	84(1)	222(4)	279(5)	198(3)
	U6	313(3)	157(10)	55(3)	81(2)	210(8)	283(3)	199(3)
	U7	300(2)	181(4)	61(1)	139(2)	256(3)	305(3)	227(4)
Turn	U8	279(4)	212(2)	55(3)	81(1)	217(4)	241(6)	199(3)
	A9	288(2)	220(3)	53(1)	148(2)	254(4)	283(6)	290(2)
D stem	2MG10	178(6)	14(11)	60(3)	89(1)	235(16)	270(15)	169(4)
	C11	303(16)	167(18)	50(3)	87(1)	211(4)	290(2)	198(2)
	U12	292(3)	175(4)	52(4)	81(1)	201(4)	296(2)	201(9)
	C13	165(8)	191(1)	180(5)	82(1)	206(14)	264(19)	191(1)
D loop	A14	87(44)	199(20)	242(34)	91(5)	235(7)	304(4)	188(6)
	G15	302(10)	165(4)	53(3)	80(3)	226(9)	215(29)	195(6)
	D16 ^b	353	91	78	97	297	225	273
	D17 ^b	30	102	176	96	177	77	219
	G18	47(23)	201(16)	58(6)	150(3)	267(3)	178(3)	261(2)
	G19	288(5)	182(5)	52(3)	154(3)	267(3)	277(3)	279(2)
	G20	277(1)	209(3)	49(4)	89(1)	239(7)	306(4)	178(3)
	A21	285(5)	147(6)	184(3)	79(2)	191(3)	284(1)	201(3)
D stem	G22	157(2)	155(5)	181(1)	84(1)	216(5)	277(5)	185(1)
	A23	309(5)	173(6)	50(1)	82(1)	201(3)	296(5)	202(1)
	G24	285(6)	182(5)	50(5)	82(1)	217(3)	289(4)	199(2)
	C25	295(4)	170(3)	56(2)	83(2)	214(3)	294(4)	199(3)
Turn	M2G26	304(6)	173(3)	49(3)	86(2)	222(2)	285(2)	198(1)
Anti-codon stem	C27	305(4)	169(2)	44(5)	83(1)	214(2)	285(4)	193(2)
	C28	292(3)	175(3)	48(2)	80(1)	210(1)	292(1)	198(6)
	A29	292(4)	170(4)	59(2)	81(1)	208(7)	280(5)	193(2)
	G30	305(7)	165(7)	58(1)	83(1)	216(8)	298(6)	190(3)
	A31	290(5)	177(7)	52(1)	83(2)	226(7)	283(8)	201(2)
Anti-codon loop	OMC32	310(7)	160(8)	47(3)	79(2)	215(4)	289(3)	208(3)
	U33	296(4)	183(5)	45(2)	83(2)	212(1)	305(1)	212(4)
	OMG34	166(7)	151(8)	54(3)	85(2)	228(3)	28(3)	186(2)
	A35	311(8)	166(7)	42(2)	80(2)	214(5)	285(4)	208(3)
	A36	288(10)	155(3)	74(7)	75(2)	203(8)	279(3)	194(3)
	YG37	303(9)	170(4)	46(4)	80(1)	218(3)	291(4)	189(2)
	A38	303(3)	173(8)	50(3)	82(1)	223(4)	283(7)	190(1)
Anti-codon stem	PSU39	308(7)	162(9)	53(4)	78(1)	221(7)	289(4)	193(2)
	5MC40	297(4)	170(3)	57(4)	82(1)	207(3)	288(1)	196(3)
	U41	292(4)	183(4)	51(2)	77(2)	223(4)	275(4)	192(1)
	G42	314(6)	157(3)	55(1)	80(1)	201(2)	288(6)	189(2)
	G43	293(6)	180(6)	57(2)	82(1)	209(4)	282(4)	199(3)
Var. loop	A44	302(10)	159(3)	61(2)	85(2)	220(1)	301(1)	200(1)
	G45	290(2)	181(5)	50(2)	87(2)	224(7)	283(6)	213(4)
	7MG46	302(8)	215(4)	47(3)	144(1)	256(6)	223(8)	296(4)
	U47	60(2)	195(5)	46(6)	14(2)	267(14)	285(15)	247(1)
	C48	287(8)	186(13)	160(4)	144(2)	215(3)	78(6)	221(2)
T stem	5MC49	49(15)	169(9)	43(9)	83(2)	215(5)	281(2)	185(2)
	U50	306(8)	180(2)	43(8)	80(2)	210(1)	292(2)	195(4)
	G51	203(5)	296(2)	176(3)	54(2)	79(1)	209(1)	290(2)
	U52	291(2)	175(4)	52(4)	80(1)	204(2)	290(5)	196(2)
	G53	303(3)	171(5)	58(1)	82(2)	202(4)	295(6)	190(2)
T loop	5MU54	283(8)	187(8)	57(5)	78(3)	231(4)	290(3)	199(4)
	PSU55	306(6)	168(3)	49(2)	75(3)	220(3)	290(3)	207(3)
	C56	166(5)	172(4)	54(3)	84(2)	228(2)	290(2)	199(2)
	G57	296(4)	167(1)	57(4)	82(1)	214(2)	294(5)	201(3)
	1MA58	297(5)	214(7)	72(7)	155(6)	280(22)	192(2)	273(2)
	U59	73(14)	202(4)	64(4)	83(5)	211(1)	308(12)	195(1)
	C60	287(4)	180(3)	66(3)	148(4)	262(7)	295(5)	242(5)

continued

TABLE 3. Continued

	Residue number	α	β	γ	δ	ϵ	ζ	χ
T stem	C61	275(15)	181(12)	39(8)	82(1)	207(4)	288(4)	193(2)
	A62	296(2)	182(4)	49(1)	80(1)	213(2)	287(2)	202(1)
	C63	298(5)	169(2)	50(3)	80(1)	206(5)	291(5)	207(3)
	A64	291(4)	181(6)	46(5)	74(2)	214(2)	285(3)	199(1)
	G65	314(7)	165(4)	52(6)	79(1)	208(4)	288(6)	187(1)
Acceptor stem	A66	300(3)	180(2)	52(3)	82(1)	211(1)	284(2)	194(3)
	A67	300(2)	161(5)	55(2)	83(1)	212(3)	279(3)	197(4)
	U68	302(1)	173(1)	47(1)	83(1)	208(1)	293(2)	199(5)
	U69	295(3)	169(2)	57(3)	58(3)	206(3)	276(2)	197(1)
	C70	297(5)	166(6)	55(1)	79(2)	203(2)	295(4)	208(1)
	G71	280(5)	174(3)	62(4)	80(2)	213(7)	291(4)	196(1)
	C72	288(4)	175(5)	64(5)	83(1)	203(1)	297(4)	199(1)
CCA end	A73	289(3)	182(1)	54(2)	82(1)	212(1)	295(3)	192(2)
	C74	292(5)	188(6)	50(1)	84(1)	207(5)	290(6)	208(5)
	C75	300(8)	194(6)	61(6)	134(0)	239(5)	89(20)	208(3)
	A76	198(49)	189(33)	245(76)	157(2)	0	0	159(19)

^aThe numbers in parentheses are the standard deviation of torsion angles of the three independently refined structures: Mn²⁺, Co²⁺, and Mg²⁺.

^bThe standard deviation values are excluded here because of the difference in conformation between the three structures at this position.

to see if the binding sites found for those ions by isomorphous difference Fourier techniques could be confirmed. The Co anomalous difference map was the more satisfactory of the two, as expected. Its three highest positive peaks, all well above noise, correspond to sites M2, M8, and M11, confirming the affinity of those sites for Co²⁺. The Co difference map also included weaker peaks corresponding to sites M9 and M10, but they could not be distinguished clearly from noise. The highest three positive peaks in the Mn anomalous difference map correspond to M2, M8, and M9, but they were all barely above noise.

Figure 5C shows the most complicated region of phenylalanine tRNA insofar as its interactions with metal ions are concerned. The M3 metal ion, which is fully hydrated by six water molecules, is located at the apex of the tight loop formed by residues 7–13. M7 and M8 form a binuclear cation cluster that interacts with the RNA at the junction of the acceptor stem and the D loop. M8, which is directly bonded to G15N7, shares a water ligand with M7, which sits between U7O2P and A14O1P. We were able to visualize only four of the six ligands expected for M7, two phosphate oxygens and two waters, but because the distances between the metal and all of those oxygens are less than 2.5 Å, we believe it is a legitimate metal-binding site. M3, M7, and M8 together stabilize the tight turn with which they are associated by neutralizing the electrostatic repulsion of its backbone phosphate groups, which are unusually close together.

The dependence of the position of nucleotide D16 on solvent divalent cation composition discussed above correlates with differences in the way the three divalent

cations in question bind to site M2. When the metal in that site is Mg²⁺, the only inner shell interaction between the metal and the RNA involves G19O2P (Fig. 7A). The remaining cation–RNA interactions, which involve D17O1P, G20N7, G20O6, and G59O4, are all mediated by water molecules. When Co²⁺ binds to M2, the geometry changes (Fig. 7C). The G19O2P interaction becomes an outer shell interaction, but the G20N7 interaction becomes inner shell. Everything else remains the same. The position assumed by the Co²⁺ is about 1.5 Å away from the position where Mg²⁺ binds, and this shift enables D16 to swing around and pair with U59. When M2 is filled with Mn²⁺ (Fig. 7B), the effect is intermediate. D16O2', which is not a ligand for either Mg²⁺ or Co²⁺, becomes an inner shell ligand, which also pulls D16 towards U59, but not quite as far.

Water-binding sites

120 water molecules were detected in this study that do not interact with metal ions. Most of them are in the molecule's major grooves and around its elbow region, which has a higher concentration of tightly bound water molecules than anywhere else in the molecule. The waters fall into three categories. Thirteen percent of the bound waters bridge sequentially adjacent phosphate oxygens. Bridging occurs not only in helical regions, as has been reported in the past (Kennard et al., 1986; Saenger et al., 1986; Leonard et al., 1994), but also in loops. Figure 6A shows the chain of water molecules that bridges phosphate groups in the TΨC loop. In helical regions, bridging waters tend to be closer to the phosphate oxygen on the 5' side than to the phosphate

FIGURE 5. (Continues on facing page.)

oxygen on the 3' side (Egli et al., 1996), but this is not always the case in loops like this one, and some of the water oxygen–phosphate oxygen distances observed in this chain are so long that one must conclude that only one of the two hydrogen bonds expected is actually formed. Ten percent of the bound waters form bridges between the 2' OH of a ribose and the phosphate oxygen on its 3' side. This motif is common in loop regions and often involve pairs of water molecules (Fig. 6B). The third class of bound waters identified, which constitutes 60% of the total, are waters that hydrogen bond directly to base donor or acceptor

groups. The positions of such waters in this structure are similar to those reported earlier by Klosterman and Steitz (Klosterman et al., 1999). It is interesting that although only 31% of the residues in this tRNA are Gs, more than 40% of the bound waters seen interact with Gs.

The regions of this structure where bound waters are most abundant are those where the temperature factors of the neighboring RNA atoms are small, and hence the structure well determined. It is likely that our failure to detect water molecules associated with high B-factor regions, such as the RNA's anticodon and CCA termi-

FIGURE 7. Stereo views of metals interacting with the D16 region. The color coding is as in Figure 5. **A:** native, Mg^{2+} structure; **B:** Mn^{2+} ; **C:** Co^{2+} .

FIGURE 8. Stereo view of the electron density in the anticodon region of the 1.93 Å resolution structure. Our interpretation of that region is shown in cyan. The 4tna interpretation is shown in magenta, and 4tra structure is depicted in green. The electron density shown is $2f_o - f_c$ density contoured at 1.0σ .

nus, has more to do with the relatively high disorder of these regions of the structure than any failure of those nucleotides to interact with water.

DISCUSSION

Comments on data collection and structure quality

In the course of this study, data were collected from many tRNA crystals, not just the three reported here. There was considerable crystal-to-crystal variation in the resolution of the data sets obtained, enough to suggest that had another 10 or 20 crystals been examined, one might have been found that diffracted better than those reported here.

As the statistics in Table 2 show, the structures reported here have good covalent geometry, but free R-factors (Brünger, 1992) that are higher than one might like. However it was not obvious from ($F_o - F_c$) difference maps or from omit maps what could or should be done to improve our structures and hence reduce their free R-factors. It is possible that the reason more satisfactory *R-free* values were not obtained is that at the resolutions of concern here, the isotropic B-factors used during refinement do not adequately model the disorder characteristic of these crystals (Correll et al., 1997, 1998; Klosterman et al., 1999). However, when anisotropic B-factors were used during refinement, no improvement resulted, probably because the ratio of data observed to parameters to be determined was not high enough.

The new structure compared to the old: Atomic positions

The simplest way to compare alternative structures for the same molecule quantitatively is to compute the rmsd between corresponding atoms after structures have been superimposed so as to minimize that difference. Table 4 gives the result of such a comparison of our final structure with the four older structures. The rmsd exceeds 1.0 Å for all four, which is substantially more than the expected error of the 1.93 Å resolution structure reported here. When D16 and D17, whose locations have been reinterpreted, are not included in both the superposition and the rmsd computation, overall rmsds fall only slightly to about 1.0 Å for the two monoclinic structures and to just under 0.9 Å for the two orthorhombic structures.

Table 4 also includes rmsds computed for specific regions of the RNA, and from these comparisons it is clear that differences in atomic positions between the structure reported here and its predecessors are concentrated in the 5' and 3' terminal regions of the RNA and in its internal loops. This conclusion is reinforced by the information shown in Figure 9, where rms dif-

TABLE 4. Rmsd between the new and the old models (Å).

	Residue number	4tna	1tra	4tra	6tna
tRNA	1–76	1.04	1.05	1.17	1.17
tRNA without residues 16–17	1–15 18–76	0.93	0.93	0.84	0.81
stem1	5–8 66–73	0.60	0.65	0.58	0.57
stem2	9–13 22–25	0.45	0.50	0.49	0.49
stem3	26–31 39–44	0.72	0.80	0.82	0.79
stem4	48–52 61–65	0.41	0.44	0.51	0.46
T loop	53–60	0.41	0.44	0.51	0.46
3',5' ends, D, AC, variable loops	1–4, 14–21 32–38 45–47 74–76	1.61	1.59	1.82	1.82

ferences in atomic position between the 1.93 Å structure and its four antecedents are presented, nucleotide by nucleotide. The peaks in all four plots centered around residues 16, 37, and 47 represent the adjustments made in the D loop, the anticodon loop, and the V loop, respectively. For comparison, Figure 9E presents the average B-factor for each nucleotide against nucleotide number. B-factors reflect the impact of both static and dynamic disorder on the structure determination process. Where B-factors are low, structure is well determined.

Figure 8 which displays the anticodon regions of the 4tna (monoclinic) structure, the 4tra (orthorhombic) structure and the new structure superimposed on the 1.93 Å resolution electron-density map, illustrates how these structures differ in that region and in many others. Clearly the three structures are interpretations of similar electron-density maps; all of them place the masses of each residue in about the same positions. The reason the new structure differs from the old is that the improved resolution of the new electron-density map demonstrates that a combination of backbone torsion angles different from those originally proposed improves the correspondence between the structure and the map.

The new structure compared to the old: Torsion angles

Table 5 is the torsion-angle equivalent of Table 4, and the message is much the same. In stem regions, most torsion angles in the 1.93 Å structure are in the same rotamer range as they are in its predecessors, but even

FIGURE 9. The dependence of rmsd in atom positions between the 1.93 Å resolution structure and its predecessors plotted against sequence position. In each graph, the average rmsd between the structure presented here and one of its predecessors is plotted residue by residue *versus* sequence position. **A:** 4tna; **B:** 1tra; **C:** 4tra; **D:** 6tna; **E:** average nucleotide B-factor plotted against sequence position. No data are reported for nt 16 and 17 where the three structures are grossly different.

TABLE 5. Torsion angle differences between the new and the old models (°).

	Residue number	4tna	1tra	4tra	6tna
tRNA	1–76	36.3	37.0	36.4	40.1
tRNA without residues 16–17	1–15 18–76	34.1	34.3	33.7	38.3
stem1	5–8 66–73	11.8	20.8	9.4	18.5
stem2	9–13 22–25	31.5	20.8	34.0	41.9
stem3	26–31 39–44	10.0	20.7	21.5	20.9
stem4	48–52 61–65	20.8	25.6	24.8	31.0
T loop	53–60	29.7	21.1	31.7	50.1
3',5' ends, D, AC, variable loops	1–4, 14–21 32–38 45–47 74–76	55.4	55.6	52.8	50.8

there the differences in torsion angles are significant compared to the errors in the current structure. For many internal loop residues, where changes in rotamer range are called for, torsion-angle differences approach 10 times error. This point is even more forcefully made by Figure 10, which displays the rms difference in torsion angles between the 1.93 Å resolution structure and each of its four predecessors, residue by residue.

The glycosidic torsion angle, χ , and the sugar pucker torsion angle, δ , are not included in the torsion-angle difference averages displayed in Figure 10 because both hardly vary from structure to structure. The sugar pucker torsion angle is either 80–85° (C3' endo) or 140–150° (C2' endo), and the only residues where there is a significant difference in δ between the 1.93 Å resolution structure and its predecessors are D16 and D17, the residues most affected by heavy metals. The glycosidic torsion angle is similarly conservative. All the residues in the old structures and the new are in the *anti*-conformation. About half of the major differences in torsion angles observed involve α and γ .

We note in passing that the conformations observed here for A21 and U47 resemble those described in the original orthorhombic crystal structures more than they do the conformations assigned them in the original monoclinic structures, even though the crystals used for this study were all monoclinic.

The new structure compared to the old: Metal-binding sites

Five of the 11 metal sites identified in this study, M1–M5, were found in the earlier crystallographic studies

FIGURE 10. The dependence of rmsd in torsion angles between the 1.93 Å resolution structure and its predecessors plotted against sequence position. In each graph, the average rmsd in torsion angles between the structure presented here and one of its predecessors is plotted residue by residue *versus* sequence position. **A:** 4tna; **B:** 1tra; **C:** 4tra; **D:** 6tna; **E:** average torsion angle standard deviations for the 1.93 Å resolution structure plotted as a function of sequence number. No data are reported for nt 16 and 17 where the three structures are grossly different.

of this RNA (Hingerty et al., 1978; Westhof & Sundaralingam, 1986; Pan et al., 1993). Three of the five, M1–M3, were identified in all four of those structures. M4 appeared to specific to the monoclinic form of tRNA, and M5 specific to the orthorhombic form of the molecule.

The preference of M2 for cations other than Mg²⁺, which is discussed above, was demonstrated in the past. It binds Zn²⁺ as well as Mn²⁺ and Co²⁺ (Jack et al., 1977; Rubin et al., 1983). These earlier studies of transition metal ion binding to yeast tRNA^{phe} also identified M8 (Jack et al., 1977), another site where our data indicate Mg²⁺ can be replaced by other divalents. In contrast to what happens at M2, when metal ions other than Mg²⁺ occupy M8, there is no change in ligand geometry. Both of the sites that bind Co²⁺ specifically, M10 and M11, involve inner sphere interactions with the N7 atoms of Gs.

The new structure compared to the old: Water sites

Water structure tends to be the most problematic aspect of a macromolecular X-ray crystal structure determination. The only part of a water molecule that is visible in all but the highest resolution electron-density maps is its oxygen. Thus in most electron-density maps, water molecules are represented by single, positive peaks that are not obviously connected to anything else. In addition, the number of such peaks an electron-density map should contain is unknown *a priori*, as are their occupancies, and both are sensitive to crystallization and data collection conditions. For all these reasons, the reliability of the water positions reported for macromolecular crystals determined at resolutions in the range of interest here are critically dependent on refinement methodology. Thus it can hardly come as a surprise that only about 10% of the water positions reported here are similar to water positions reported earlier, and that the identity of the 10% which is similar depends on which of the older structures one consults. One reason for being confident that the water positions reported here are reliable is that they conform in large measure to placements reported in RNA structures determined at much higher resolution, as discussed earlier.

MATERIALS AND METHODS

Purification and crystallization

Phenylalanine tRNA derived from *Saccharomyces cerevisiae* was purchased from Sigma. It is heterogeneous because *S. cerevisiae* has two genes for phenylalanine tRNAs, both of which are expressed (Venegas et al., 1981). In the less abundant of the two, the U₆-A₆₇ base pair in the acceptor stem of the more abundant form (Fig. 1) is replaced by a C₆-G₆₇ base pair (Keith & Dirheimer, 1987). The two isoacceptors were

separated electrophoretically on 10% polyacrylamide gels in .045 M tris-borate, 1 mM EDTA, pH 8, at 4 °C, and their difference in sequence confirmed. Transfer RNA was recovered from gels by electroelution, dialyzed against 2 mM MgCl₂, and then precipitation with ethanol. Precipitates were dried in a desiccator at 4 °C, and then dissolved in water at a concentration of 4 mg/mL. Crystals were produced by the hanging-drop method following earlier protocols (Kim et al., 1973). The reservoir solution was 7–9% isopropanol in 40 mM MgCl₂, 3 mM spermine, 40 mM sodium cacodylate (pH 6–7) and the temperature was 4 °C. Drops contained equal amounts of tRNA and reservoir solution. The best crystals resulted when trays were left at room temperature for a few hours before being transferred to incubators at 4 °C. They were thin plates, 0.25–0.5 mm in their largest dimension. Crystals were stabilized by slowly adding concentrated buffer solutions to the drops in which they were grown. For most crystals, the final buffer composition was: 40 mM MgCl₂, 30 mM spermine, 25% MPD, 18% glycerol, 40 mM sodium cacodylate (pH 6–7). The structure that emerged from the analysis of crystals stabilized this way is referred to below as the “native” structure. Some crystals were stabilized in a similar manner using stabilizing buffers to which either MnCl₂ or CoCl₂ had been added. The structures these crystals gave are referred to below as the “Mn²⁺” and “Co²⁺” structures, respectively. The final concentration of Mn²⁺ or Co²⁺ in these crystals was 2 mM. All crystals were flash frozen in liquid propane at liquid N₂ temperature prior to data collection.

Data collection and analysis

Native data were collected at Brookhaven National Laboratory on beamline X12C, using a Brandeis 2 × 2 CCD detector. Data sets were obtained from Mn²⁺- and Co²⁺-soaked crystals on beamline A1 at the Cornell High Energy Synchrotron Source using a Q4 2 × 2 CCD detector. Data sets were processed using DENZO and SCALEPACK (Otwinowski & Minor, 1997). During data collection, there was a slow build-up of ice on the Mn²⁺-soaked and the Co²⁺-soaked crystals examined. This resulted in the development of ice rings in diffraction patterns obtained. We were unable to find a way to correct reflections that have ice-ring spacings for the background generated by ice diffraction, and consequently did not include data for such reflections in the data sets analyzed.

The structures of these crystals were solved by molecular replacement using the 4tna structure as the starting model. The first step was rigid-body refinement of the placement of the 4tna structure in the unit cell, which resulted in a minor adjustment of its position. The structure was then refined by simulated annealing using the torsion-angle algorithm in the crystallography and NMR system (CNS) (Brünger et al., 1998, 1990; Rice & Brünger, 1994). The phases computed from the refined model were combined with the observed amplitudes to generate a new electron-density map, on the basis of which the structure was adjusted by hand and the positions of bound water molecules and metal ions determined (see below). Several additional cycles of refinement and structure rebuilding followed. Heavy use was made of composite omit maps to eliminate model bias. Electron-density maps were interpreted using the graphics display program O (Jones et al., 1991).

Peaks in the electron-density map not accounted for by the covalent structure of the RNA were interpreted as water

molecules provided: (1) that their heights exceeded 2.5 σ , (2) that they were located 2.5–3.5 Å away from hydrogen bond donors or acceptors, and (3) that their B-factors refined to less than 60 Å². Heavy-metal sites were modeled by fitting metal ions with ideal octahedral ligand geometry into omit maps, and then refining with inner-sphere bond lengths fixed at 2.0 Å.

We note in closing that Dr. Daniela Rhodes and her colleagues have also redetermined the crystal structure of the yeast tRNA^{Phe} recently [ref.].

ACKNOWLEDGMENTS

We acknowledge the contribution to this study of John Drenth, who grew the first phenylalanine tRNA crystals in this laboratory. We thank Jimin Wang and Poul Nissen for their valuable advice, the members of the group of Thomas Steitz for their help with data collection, and the staff of the Yale Center for Structural Biology for their assistance with data processing. The data on which this study is based were collected at the National Synchrotron Light Source (Brookhaven) and at the Cornell High Energy Synchrotron Source. This work was supported by a grant from the National Institutes of Health (GM 54216).

Received February 17, 2000; returned for revision March 14, 2000; revised manuscript received May 10, 2000

REFERENCES

- Brünger AT. 1992. The free R value: A novel statistical quantity for assessing the accuracy of crystal structures. *Nature* 355:472–474.
- Brünger AT, Adams PD, Clore GM, Delano WL, Gros P, Grosse-Kunstleve RW, Jiang J-S, Kuszewski J, Nilges N, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. 1998. Crystallography and NMR system (CNS): A new software system for macromolecular structure determination. *Acta Cryst D* 54:905–921.
- Brünger AT, Krukowski A, Erickson J. 1990. Slow-cooling protocols for crystallographic refinement by simulated annealing. *Acta Cryst A* 46:585–593.
- Carson M. 1991. Ribbon 2.0. *J Appl Cryst* 24:958–961.
- Christopher JA. 1998. *SPOCK: The structure properties observation and calculation kit* (program manual). College Station, Texas: The Center for Macromolecular Design, Texas A&M University.
- Correll CC, Freeborn B, Moore PB, Steitz TA. 1997. Metals, motifs and recognition in the crystal structure of a 5S rRNA domain. *Cell* 91:705–712.
- Correll CC, Munishkin A, Chan YL, Ren Z, Wool IG, Steitz TA. 1998. Crystal structure of the ribosomal RNA domain essential for binding elongation factors. *Proc Natl Acad Sci USA* 95:13436–13441.
- Egli M, Portmann S, Usman N. 1996. RNA hydration: A detailed look. *Biochemistry* 35:8489–8494.
- Hingerty B, Brown RS, Jack A. 1978. Further refinement of the structure of yeast tRNA. *J Mol Biol* 124:523–534.
- Jack A, Ladner JE, Rhodes D, Brown RS, Klug A. 1977. A crystallographic study of metal-binding to yeast phenylalanine transfer RNA. *J Mol Biol* 111:315–328.
- Jones TA, Zou J-Y, Cowan SW, Kjeldgaard M. 1991. Improved methods for building models in electron density maps and the location of errors in these models. *Acta Cryst A* 47:110–119.
- Keith G, Dirheimer G. 1987. Evidence for the existence of an expressed minor variant tRNA^{Phe} in yeast. *Biochem Biophys Res Comm* 142:183–187.
- Kennard O, Cruse WB, Nachman J, Prange T, Shakked Z, Rabinovich D. 1986. Ordered water structure in an A-DNA octamer at 1.7 Å resolution. *J Biomol Struct Dyn* 3:623–647.
- Kim SH, Quigley G, Suddath FL, McPherson A, Sneden D, Kim JJ,

- Weinzierl J, Rich A. 1973. X-ray crystallographic studies of polymorphic forms of yeast phenylalanine transfer RNA. *J Mol Biol* 75:421–428.
- Kim SH, Suddath FL, Quigley GJ, McPherson A, Sussan JL, Wang AHJ, Seeman NC, Rich A. 1974. Three-dimensional tertiary structure of yeast phenylalanine transfer RNA. *Science* 185:435–440.
- Klosterman PS, Shah SA, Steitz TA. 1999. Crystal structures of two plasmid copy control related RNA duplexes: An 18 base pair duplex at 1.20 Å resolution and a 19 base pair duplex at 1.55 Å resolution. *Biochemistry* 38:14784–14792.
- Leonard GA, McAuley-Hecht KE, Ebel S, Lough DM, Brown T, Hunter WN. 1994. Crystal and molecular structure of r(CGCGAAUU AGCG): An RNA duplex containing two G(anti).A(anti) base-pairs. *Structure* 2:483–494.
- Luzzati PV. 1952. Traitement Statistique des erreurs dans la détermination des structures cristallines. *Acta Cryst* 5:802–810.
- Otwinowski Z, Minor W. 1997. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 276:307–326.
- Pan T, Long DM, Uhlenbeck OC. 1993. Divalent metal ions in RNA folding and catalysis. In: Gesteland RF, Atkins JF, eds. *The RNA world*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 271–302.
- Pley HW, Flaherty KM, McKay DB. 1994. Three-dimensional structure of a hammerhead ribozyme. *Nature* 372:68–74.
- Rice LM, Brünger AT. 1994. Torsion angle dynamics: Reduced variable conformational sampling enhances crystallographic structure refinement. *Proteins* 19:277–290.
- Robertus JD, Ladner JE, Finch JT, Rhodes D, Brown RS, Clark BFC, Klug A. 1974. Structure of yeast phenylalanine tRNA at 3 Å resolution. *Nature* 250:546–551.
- Rubin JR, Wang J, Sundaralingam M. 1983. X-ray diffraction study of the zinc(II) binding sites in yeast phenylalanine transfer RNA. *Biochim Biophys Acta* 756:111–118.
- Saenger W. 1984. *Principles of nucleic acid structure*. New York: Springer-Verlag.
- Saenger W, Hunter WN, Kennard O. 1986. DNA conformation is determined by economics in the hydration of phosphated groups. *Nature* 324:385–388.
- Stout CD, Mizuno H, Rubin J, Brennan T, Rao ST, Sundaralingam M. 1976. Atomic coordinates and molecular conformation of yeast phenylalanyl tRNA: An independent investigation. *Nucleic Acids Res* 3:1111–1123.
- Sussman JL, Holbrook SR, Warrant RW, Church GM, Kim S-H. 1978. Crystal structure of yeast phenyl alanine transfer RNA I. Crystallographic refinement. *J Mol Biol* 123:607–630.
- Venegas A, Bello A, Villanueva J, Zaldivar J, Quiroga M, Gomez I, Bull P, Valenzuela P. 1981. Isolation and structure of the Yeast tRNA genes. In: Siddiqui MAQ, Krauskopf M, Weissbach H, eds. *Molecular approaches to gene expression and protein structure*. New York: Academic Press.
- Westhof E, Dumas P, Moras D. 1988. Restrained refinement of two crystalline forms of yeast aspartic acid and phenylalanine transfer RNA crystals. *Acta Cryst A* 44:112–123.
- Westhof E, Sundaralingam M. 1986. Restrained refinement of the monoclinic form of yeast phenylalanine transfer RNA. Temperature factors and dynamics, coordinated waters, and base-pair propeller twist angles. *Biochemistry* 25:4868–4878.