The Binding of Complementary Oligoribonucleotides to Yeast Initiator Transfer RNA†

Susan M. Freier* and Ignacio Tinoco, Jr.

ABSTRACT: Oligoribonucleotide binding to baker's yeast initiator tRNA was measured by equilibrium dialysis in order to determine which regions of the tRNA were free to bind complementary oligomers and which were involved in secondary and tertiary structure. Association constants of trinucleoside diphosphates and tetranucleoside triphosphates complementary to the single-stranded regions of the cloverleaf structure of yeast $tRNA^{fMet}$ were measured at 0° in 1.0 *M* NaCl, and 0.01 *M* $MgCl_2$. The only regions of the tRNA whose complementary oligomers bound to the tRNA were the amino acid acceptor end and the five nucleotides at the 5′ end of the anticodon loop. These results differ from those for the other tRNAs studied by this technique; usually oligomers complementary to the dihydrouracil loop bind to the tRNA. The sequence of yeast $tRNA^{fMet}$ and other eucaryotic initiators is unusual. The "TψC loop" contains the sequence A-U-C instead of T-ψ-C, yet the binding pattern to the TψC loop is like that for other tRNAs; no oligomers bind.

The structures of tRNAs have been studied extensively. Very little is known, however, about the tertiary structure of yeast $tRNA^{fMet}$ although, as a eucaryotic initiator, it plays a unique role in protein synthesis. For that reason yeast initiator tRNA was studied by oligonucleotide binding. We thought its unique biological role might be reflected by unique molecular structure. X-Ray diffraction studies are being made on crystals of this molecule by Dr. Paul Sigler and his colleagues. We plan to compare the solution structure of this molecule with its crystal structure.

The measurement of association constants of oligoribonucleotides to tRNA has previously been used extensively to study the secondary and tertiary structure of only four tRNAs.[1] Oligomers complementary to exposed single-stranded regions of the tRNA are expected to bind strongly and specifically to the tRNA. Nonbinding oligomers complementary to the tRNA are assumed to be complementary to regions involved in secondary and tertiary folding. Oligomers not complementary to the tRNA are not expected to bind.

Materials and Methods

Transfer RNA. Yeast $tRNA^{fMet}$ was purified from mixed baker's yeast tRNA (Plenum) according to the procedure of Pasek et al. (1973). Using a standard assay procedure (RajBhandary and Ghosh, 1969), the purified tRNA accepted 1.7 nmol of methionine/A_{260} unit and was judged to be at least 85% pure.

Oligoribonucleotides. Many of the tetranucleoside triphosphates were a generous gift of Professor Olke Uhlenbeck. The other tritiated oligonucleotides were synthesized using primer dependent polynucleotide phosphorylase from *Micrococus luteus* according to the procedure of Uhlenbeck et al. (1970). The primer dependent polynucleotide phosphorylase was purified from a partial trypsin digest of primer independent polynucleotide phosphorylase (P-L Biochemicals). The specific activity of the oligonucleotides was 6–12 Ci/mol.

Equilibrium Dialysis Experiments. Equilibrium dialysis experiments were performed in two 50-μl plexiglass chambers with a section of dialysis tubing sealed between them. One chamber was loaded with about 1.3 A_{260} units of tRNA dissolved in 1 *M* NaCl–0.01 *M* $MgCl_2$–0.01 *M* sodium phosphate (pH 7.0). The other chamber was filled with about 0.25 μCi of tritiated oligomer in the same buffer. The dialysis cell was stored at 0° for 3–7 days to allow for equilibration; 3μl aliquots were extracted in triplicate from each chamber on each of three consecutive days. Results did not change from day to day indicating equilibrium had been achieved. The aliquots were diluted with 0.1 ml of water and counted by liquid scintillation in a toluene based scintillation fluid (5 g of 2,5-diphenyloxazole/l. (Amersham) and 2.5% Biosolve (Beckman)).

Calculation of Equilibrium Constants. An association constant (K) was calculated from the ratio (R) of counts in the chamber containing the tRNA to the counts in the chamber without tRNA according to the relation $R = 1 + K[\text{tRNA}]$. This relation is valid when the total tRNA concentration is much larger than the total oligomer concentration. For most measurements, the tRNA concentration was 49 μ*M* and the oligomer concentration was less than 1 μ*M*. The validity of this relation over a tRNA concentration range 20–200 μ*M* was verified for one trimer (see Results section).

Repetitions of experiments using different preparations of oligomer and tRNA gave binding constants differing by up to 20%.

In calculating equilibrium constants from the equilibrium dialysis data, the tRNA was assumed to be pure; no corrections were made for impurities.

Results

Association constants of trinucleoside diphosphates and tetranucleoside triphosphates to yeast $tRNA^{fMet}$ are listed in Table II. For oligomers containing GpG residues, the experiment was done in 0.001 *M* EDTA instead of 0.01 *M* $MgCl_2$ to reduce oligomer aggregation and reduce equili-

† From the Department of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley, California 94720. *Received March 3, 1975.* This investigation is supported in part by Grant GM 10840 and by the U.S.A.E.C.

[1] References on oligonucleotide binding to tRNAs are listed in Table I.

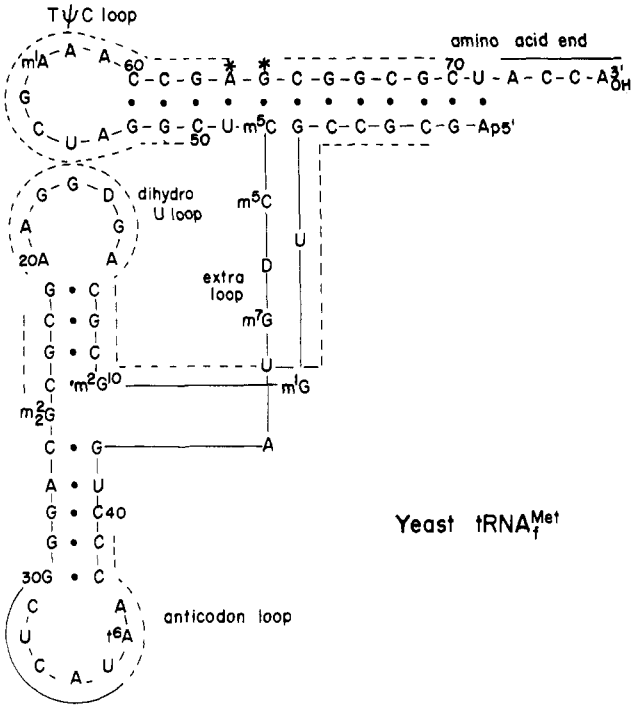

FIGURE 1: Nucleotide sequence of yeast tRNA^{fMet} (Simsek and RajBhandary, 1972). Symbols for the unusual nucleosides are m^{1}G = 1-methylguanosine, m^{2}G = N^2-methylguanosine, D = 5,6-dihydrouridine, m$_2^2$G = N^2,N^2-dimethylguanosine, t^{6}A = N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine, m^{7}G = 7-methylguanosine, m^{5}C = 5-methylcytosine, m^{1}A = 1-methyladenosine. A* and G* are modified nucleosides whose structure has not yet been established. Solid lines indicate regions whose complementary oligomers bound to the tRNA; dashed lines indicate regions complementary to oligomers that did not bind.

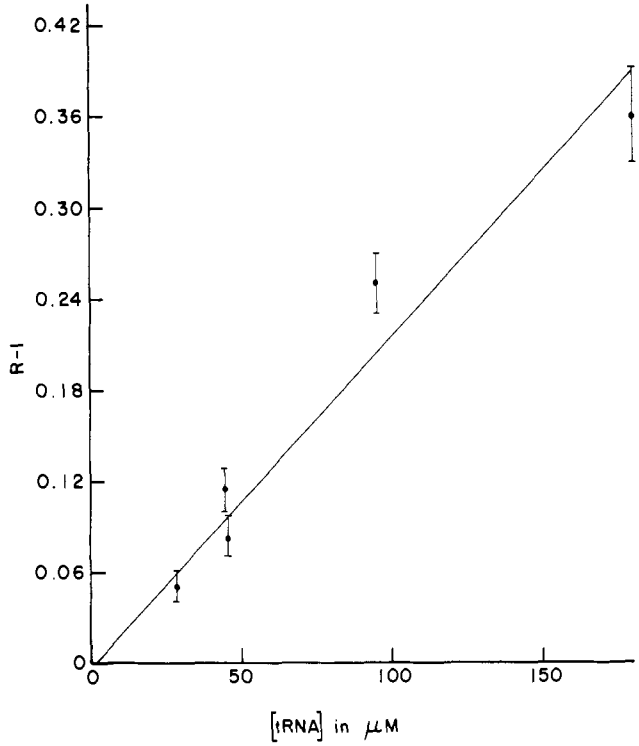

FIGURE 2: Plot of $R - 1$ vs. tRNA concentration for A-U-G binding to yeast tRNA^{fMet}. R is the ratio of counts in the chamber containing the tRNA to the counts in the chamber containing only oligonucleotide. The slope of the line gives $K = 2000\ M^{-1}$ which agrees, within experimental error, with the value obtained at a tRNA concentration of 50 μM.

bration times. The error in the association constants due to sampling and counting errors is ±500. Therefore trimers with binding constants less than 500 are listed as not binding.

The region of yeast initiator tRNA complementary to each oligomer is also tabulated. Figure 1 shows the sequence of tRNA^{fMet} (Simsek and RajBhandary, 1972). Regions complementary to oligomers which did not bind are marked by dashed lines; solid lines indicate regions where complementary oligomers did bind to the tRNA. The significance of these results and the conclusions about tRNA structure that can be made from them are discussed below.

For the codon, A-U-G, the dependence of the measured association constant on tRNA concentration was determined. Figure 2 presents a graph of $R - 1$ vs. tRNA concentration for A-U-G binding to yeast tRNA^{fMet} over a tRNA concentration range 20–200 μM. R is the ratio of counts in the chamber containing the tRNA to counts in the chamber containing only oligonucleotide. Over this concentration range the graph is linear indicating no detectable tRNA aggregation.

The association constants of oligomers complementary to the amino acid acceptor end of the tRNA were all measured in the absence of magnesium. It had been assumed that at high salt concentrations (1 M NaCl) the presence or absence of 0.01 M Mg^{2+} would not greatly affect the association constants. This assumption was tested by measuring the association constant of A-U-G in both the EDTA buffer and the magnesium buffer. The association constant in the absence of Mg^{2+} is 4000 M^{-1}, while in the presence of Mg^{2+} it is only 2000 M^{-1}. It is not known whether this increase in association constant upon removal of Mg^{2+} is due to changes in tRNA structure, trimer conformation, or the nature of the interaction between the two. This apparent sensitivity of the tRNA–oligomer association to the concentration of Mg^{2+} even in the presence of high Na^+ concentrations will be studied further.

Discussion

Function of Yeast tRNA^{fMet}. Biologically, yeast initiator tRNA differs from both noninitiator yeast tRNAs and *E. coli* initiator tRNA. Yeast tRNA^{fMet} undergoes a number of specific interactions with initiation factors, the ribosomes, and mRNA during the initiation of protein synthesis in yeast. (For a review of protein synthesis in eucaryotes see Haselkorn and Rothman-Denes, 1973.) Yeast tRNA can be formylated in vitro using the *Escherichia coli* formyl donor and formylating enzyme. In vivo, however, it is used as unformylated Met-tRNA^{fMet} to initiate protein synthesis. The specificity of the interactions of yeast initiator tRNA with other cellular molecules suggests a unique structure of tRNA^{fMet} so that it can be distinguished from other noninitiator tRNAs.

Comparison to Binding Patterns of Other tRNAs. These binding studies reveal both similarities and differences between yeast tRNA^{fMet} and other tRNAs studied by this technique. (See Table I for a summary of other tRNAs studied.) It appears the secondary structure of yeast tRNA^{fMet} is the cloverleaf; only oligomers complementary to single-stranded regions of the cloverleaf are among those judged to bind to the tRNA. The binding pattern of the loops, however, is different from that of other tRNAs, possibly reflecting unusual features in the folding of this tRNA.

Table I: Summary of References Giving Association Constants of Oligoribonucleotides to tRNA.[a]

Ref	tRNA Studied
Cameron and Uhlenbeck (1973)	Yeast $tRNA^{Phe}$
Eisinger et al. (1971)[b]	Yeast $tRNA^{Phe}$
Eisinger and Spahr (1973)[c]	Yeast $tRNA^{Phe}$
Högenauer (1970)	*E. coli* $tRNA^{Met}_m$ + $tRNA^{fMet}$, *E. coli* $tRNA^{fMet}$
Högenauer et al. (1972)	*E. coli* $tRNA^{Met}_m$, yeast $tRNA^{fMet}$, *E. coli* $tRNA^{fMet}$
Miller et al. (1974)	Yeast $tRNA^{Phe}$, *E. coli* $tRNA^{Phe}$
Pongs et al. (1971)	Yeast $tRNA^{Phe}$
Pongs et al. (1973)	Yeast $tRNA^{Phe}$
Pongs and Reinwald (1973)	Yeast $tRNA^{Phe}$
Schimmel et al. (1972)	*E. coli* $tRNA^{Ile}$
Uhlenbeck (1972)	*E. coli* $tRNA^{Tyr}$, *E. coli* $tRNA^{fMet}$
Uhlenbeck et al. (1970)	*E. coli* $tRNA^{fMet}$
Uhlenbeck et al. (1974)	Yeast $tRNA^{Leu}_3$

[a] The association constants were measured by equilibrium dialysis unless otherwise noted. [b] Changes in Y base fluorescence were used to measure binding. [c] Gel electrophoresis was used to measure binding.

Table II: Association Constants of Oligoribonucleotides to Yeast $tRNA^{fMet}$.[a]

	K (M^{-1})	Complementary Regions of tRNA[b]
Oligomers complementary to dihydrouracil loop		
C-C-A-C	<500	15–18 (7–10)
U-C-C	<500	17–19 (51–53)
U-U-C	<500	18–20
U-U-C-C	<500	17–20
Oligomers complementary to anticodon loop		
G-A-G	3,300	31–33 (48–50)
U-G-A	<500	32–34
U-G-A-G	5,000	31–34
A-U-G	2,000	33–35
A-U-G-A	6,200	32–35
U-A-U-G	500	33–36
A-U-G	4,000[c]	33–35
Oligomers containing wobble codon		
G-U-G	1,900	33–35
G-U-G-A	8,000	32–35
Oligomers complementary to TψC loop		
G-A-U	<500	53–55
U-C-G	<500	55–57 (61–63)
U-C-G-A	1,300	54–57
U-U-U-C	<500	56–59
U-U-C	<500	56–58
Oligomers complementary to amino acid acceptor end		
U-G-G	1,800[c]	73–75
G-G-U	40,000[c]	72–74 (37–39)
U-G-G-U	30,000[c]	72–75 (59–61)
Others		
G-C-G	1,200	(3–5)(11–13)(22–24)(68–70)
C-C-G	2,500	(65–67)(50–52)
G-G-C	20,000[c]	(4–6)
A-C-G	<500	None
U-U-G-U	<500	None
C-U-G	<500	(26–28)(13–15)
G-U-U	<500	(36–38)(58–60)
A-U-G-C	1,200	None

[a] Association constants were measured at 0°. 1 M NaCl–0.01 M MgCl–0.01 M sodium phosphate (pH 7.0). [b] Regions enclosed in parentheses are contained at least partially in one of the four double helical stems of the tRNA cloverleaf. [c] Association constants of oligomers containing the sequence GpG were measured at 0°, 1 M NaCl–0.001 M EDTA–0.01 M sodium phosphate (pH 7.0).

The dihydrouracil loop of yeast $tRNA^{fMet}$ binds to none of its complementary oligomers. These results differ from those for yeast $tRNA^{Phe}$, yeast $tRNA^{Leu}_3$, *E. coli* $tRNA^{fMet}$, and *E. coli* $tRNA^{Tyr}$ where oligomers complementary to the dihydrouracil loop do bind to the tRNA.[1] The dihydrouracil loop of yeast initiator tRNA contains only seven bases; the range of dihydrouracil loop sizes among sequenced tRNAs is 7–12 bases. The seven-membered anticodon loop, however, binds its complementary oligomers, so the small size of the dihydrouracil loop cannot be the only reason its complementary oligomers do not bind the tRNA. The nucleotide dihydrouridine forms only very weak base pairs (Cerutti et al., 1966) so it is not unexpected that C-C-A-C complementary to G-D-G-G_{15-18} does not bind strongly to the tRNA. If the loop were free, however, one would expect U-U-C, U-C-C, and U-U-C-C to bind to the G-G-A-A_{17-20} region with an association constant of about 1000 M^{-1} which would be easily detected by this technique. The fact that these oligomers do not bind indicates this loop is involved in tertiary folding.

The binding pattern to the anticodon loop is very similar to that for other tRNAs studied.[1] The five nucleotides at the 5′ end of the loop bind to their complementary oligomers while the two bases on the 3′ end are apparently unavailable for binding. One of these two bases is a hypermodified adenine, t^6A. This nucleotide may prevent oligomers complementary to the nucleotides at the 3′ end of the anticodon loop from binding to the tRNA.

The sequence of the anticodon loop of *E. coli* $tRNA^{fMet}$ is nearly identical with that of the anticodon loop of yeast $tRNA^{fMet}$. *E. coli* $tRNA^{fMet}$ has an unmodified adenosine on the 3′ end of the anticodon, but in the yeast species that adenosine has a threonyl hypermodification at N^6. The binding pattern to the anticodon loop of *E. coli* $tRNA^{fMet}$ is compared to that of yeast $tRNA^{fMet}$ in Table III. There are no outstanding differences between the two indicating the structures of the two anticodon loops are similar, in spite of the hypermodified t^6A in the yeast tRNA. The hypermodification apparently does not drastically affect codon or wobble codon binding. The binding of A-U-G to yeast $tRNA^{fMet}$ and *E. coli* $tRNA^{fMet}$ and *E. coli* $tRNA^{Met}_m$ was measured by Högenauer et al. (1972) under slightly different conditions. Although these association constants differ greatly from those in Table II, the same conclusions were drawn; the t^6A does not seem to affect codon binding.

It is interesting to note the wobble codon binding is as efficient as codon binding. Both G-U-G and G-U-G-A bind to the tRNA as strongly as A-U-G and A-U-G-A, respectively, indicating the G·U base pair at the 3′ end of the anticodon is as strong as an A·U pair.

Degeneracy in the genetic code occurs for noninitiation codons primarily in the nucleotide at the 3′ end of the codon. The initiator codon is unique; however, its degeneracy occurs in the nucleotide at the 5′ end of the codon. Both A-U-G and G-U-G code for initiation. Apparently for noninitiator tRNA–codon complexes, a G·U base pair results in a stable complex only if the mismatch is between

Table III: Association Constants of Oligomers Complementary to the Anticodon Loops in *E. coli* and Baker's Yeast Initiator tRNAs.[a]

Oligomer	*K* for *E. coli* $tRNA^{fMet}$ (in M^{-1})	*K* for yeast $tRNA^{fMet}$ (in M^{-1})
G-A-G	1,000	3300 ± 500
U-G-A	400	
A-U-G	1,200	2000
G-U-G	1,200	1900
A-U-G-A	13,500	6200
G-U-G-A	9,800	8000
U-G-A-G	2,800	5000
A-U-G-C	–[b]	–[b]
U-A-U-G	–[b]	–[b]

[a] The conditions are the same as given in Table I. Data for *E. coli* $tRNA^{fMet}$ are taken from Uhlenbeck (1972). [b] Tetramer association constants marked with – are judged not binding as tetramers because the association constant of the tetramer is less than twice the sum of the association constants of its constituent trimers. Presumably, only three of the four nucleotides in the tetramer are binding directly to the tRNA.

the nucleotide at the 5′ end of the anticodon and the nucleotide at the 3′ end of the codon. With initiator tRNAs, however, a G·U mismatch at the other end of the codon is allowed.

The degeneracy in the genetic code, in the nucleotide at either the 3′ or the 5′ end of the codon, is always the same degeneracy seen in oligonucleotide binding experiments. In *E. coli* $tRNA^{fMet}$ the unusual wobble at the 5′ end of the codon was attributed to the absence of a t^6A hypermodified nucleotide adjacent to the anticodon (Dube et al., 1968). All presently sequenced noninitiator tRNAs with a U at the 3′ end of the anticodon have a t^6A next to the anticodon. The triplet that codes for the amino acid of each of these tRNAs is AXY, where X and Y are any of the four nucleotides. GXY, the corresponding triplet which would bind to the anticodon with a G·U base pair at the 3′ end of the anticodon, does not code for the same amino acid. This suggested that the role of the t^6A adjacent to the anticodon was to prevent a G·U mismatch at the 3′ end of the codon. The present results disprove this speculation. Both G-U-G and A-U-G bind equally well to yeast $tRNA^{fMet}$ in spite of the presence of the t^6A nucleotide.

Stewart et al. (1971) demonstrated that a mutation A-U-G to G-U-G at the initiation site of iso-1-cytochrome *c* in yeast prevents synthesis of the protein. They then concluded G-U-G is not recognized in vivo as an initiation codon in yeast. The present results demonstrate G-U-G does bind to yeast initiator tRNA. The fact that G-U-G does not initiate protein synthesis in Stewart's mutant cannot be attributed to poor G-U-G·$tRNA^{fMet}$ association.

Perhaps the most interesting results are for the TψC loop. Eucaryotic initiator tRNAs are unique; in five which have been studied, the sequence T-ψ-C-G is replaced by A-U-C-G (Simsek et al., 1973; Piper and Clark, 1973). Whatever role these modified bases play in the functioning of noninitiator tRNAs and *E. coli* initiator tRNA, they are unnecessary for the functioning of yeast initiator tRNA. The sequence of yeast initiator tRNA is also unusual at position 59 where there is an adenosine; in all other sequenced tRNAs there is a pyrimidine at the 3′ end of the TψC loop. This unusual sequence of the TψC loop does not affect binding at all. Like *E. coli* $tRNA^{fMet}$ and *E. coli* $tRNA^{Tyr}$ (Uhlenbeck, 1972) no oligomers complementary to the TψC loop bind to yeast $tRNA^{fMet}$. Even without TψC it appears the TψC loop is buried in tertiary structure and unavailable to bind its complementary oligomers.

The three oligomers complementary to the amino acid acceptor end all bind to $tRNA^{fMet}$ from yeast about half as strongly as to other tRNAs.[1] This may be because the amino acid acceptor end of yeast initiator tRNA is involved in tertiary structure. It is also interesting to note the -C-C-A end of many tRNAs is easily hydrolyzed during purification or other handling of the tRNA. This is evidenced by the fact that the addition of both CTP and ATP to the incubation mixture when aminoacylating many yeast tRNAs increases the amino acid acceptance of the tRNA over that when only ATP is added. The -C-C-A end of yeast $tRNA^{fMet}$ is relatively stable and remains intact under conditions where other tRNAs lose their -C-C-A. Preincubation of yeast $tRNA^{fMet}$ with CTP, ATP, and crude *E. coli* protein extract does not increase its methionine acceptance activity.

The two trimers U-G-G and G-G-U both have the same base composition and very similar nearest neighbor interactions yet their association constants to the amino acid acceptor end of the tRNA are greatly different. According to the cloverleaf model, the amino acid acceptor end is a free single-stranded end. We expect the binding of oligomers to it to be different from the binding to tRNA loops. The trimer U-G-G is complementary to $C\text{-}C\text{-}A_{73\text{-}75}$, the end three bases, while G-G-U is complementary to the $A\text{-}C\text{-}C_{72\text{-}74}$, adjacent to the double helical region of the amino acid acceptor end. The fact that G-G-U binds so much more strongly than U-G-G is probably because the G-G-U forms a helix with $A\text{-}C\text{-}C_{72\text{-}74}$ which can stack on the amino acid acceptor end helix forming one continuous helix. The three base pair helix that U-G-G forms with $C\text{-}C\text{-}A_{73\text{-}75}$ is one base removed from the double helical stem so it cannot stack on that stem. Also, we expect the base pair at the end of a chain to be less stable than interior base pairs (Levine, 1974).

The difference between the association constants of G-G-U and U-G-G to yeast $tRNA^{fMet}$ may not reflect tRNA structure. The binding of oligoribonucleotides to synthetic RNA copolymers was measured by Lewis et al. (1975). The measured association constant of U-G-G to poly($A_{4.6}$,C) is 15,100 M^{-1}, corrected for site concentration of the complementary sequence assuming random copolymerization. Under the same conditions, 0°, 1 *M* NaCl–0.005 *M* EDTA–0.01 *M* cacodylate (pH 7.2), G-G-U binds to poly($A_{4.6}$,C) with an association constant of 90,000 M^{-1}. This indicates the stronger binding of G-G-U to yeast $tRNA^{fMet}$ than that of U-G-G may be due only to sequence differences and not differences in the structure of the complementary site caused by tRNA conformation.

Comparison of Results with Crystal Structure. A detailed model of the tertiary structure of yeast $tRNA^{Phe}$ based on X-ray crystal diffraction data has been proposed by Kim et al. (1974a) and Robertus et al. (1974). Based on this model of yeast $tRNA^{Phe}$, Kim et al. (1974b) have proposed a general model of the structure of tRNA. The sequence of yeast $tRNA^{fMet}$ is similar enough to that of yeast $tRNA^{Phe}$ that tertiary structure base pairs proposed for $tRNA^{Phe}$ are also possible for yeast $tRNA^{fMet}$. The tertiary structure base pairs necessary to fit yeast $tRNA^{fMet}$ to the yeast $tRNA^{Phe}$ tertiary structure are $G_7\cdot C_{22}\cdot G_{12}$, $U_8\cdot A_{14}\cdot A_{20}$, $G_{15}\cdot m^5G_{47}$, $G_{17}\cdot U_{54}$, $G_{18}\cdot C_{55}$, $G_{21}\cdot m^7G_{45}$, and $m^2G_{25}\cdot A_{43}$. A tertiary structure for yeast $tRNA^{fMet}$ can therefore be imagined similar to the crystal structure of

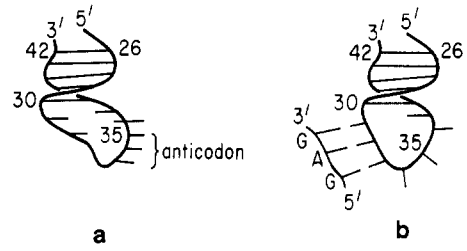

FIGURE 3: (a) A schematic drawing of the anticodon loop of yeast $tRNA^{fMet}$ according to the model of Robertus et al. (1974). With C_{31} and U_{32} stacked on the inside of the loop there is no room for the trimer G-A-G to fit inside the loop and bind to C-U-C_{31-33}. (b) By rotating the bases C_{31} and U_{32} to the outside of the loop and changing the conformation of the loop slightly it is possible to bind G-A-G to the tRNA.

yeast $tRNA^{Phe}$. Are the binding data consistent with such a structure?

According to the Kim and Robertus structure, only D_{16} and A_{19} in the dihydrouracil loop are not hydrogen bonded. The nonbinding of all oligomers complementary to the dihydrouracil loop supports this model while the binding of oligomers complementary to the dihydrouracil loop in other tRNAs is not consistent with the model. In the TψC loop, the X-ray data support a model where all of the bases are sheltered, either base paired or involved in internal stacking interactions. Again, the binding results are consistent with this model. For the anticodon loop, the yeast $tRNA^{Phe}$ crystal model is incompatible with the binding results. Applying the model of Kim et al. (1974a,b) and Robertus et al. (1974) to the anticodon loop of yeast $tRNA^{fMet}$, there would be no room for the trimer G-A-G to bind to C-U-C_{31-33}. This is illustrated in Figure 3a. However, a slight modification of the structure would allow G-A-G to bind. This is illustrated in Figure 3b. The fact that the tetramers A-U-G-A and U-G-A-G both bind to the tRNA either supports a model of the anticodon loop which is flexible, or indicates the crystal structure is not identical with that in solution. Binding of U-U-C-A-G to yeast $tRNA^{Phe}$ (Eisinger and Spahr, 1973) is also consistent with this conclusion.

A schematic drawing of yeast $tRNA^{fMet}$ in the L configuration is shown in Figure 1. It is clear that the binding results are consistent with this overall shape.

Conclusions

Yeast $tRNA^{fMet}$ is functionally unique from other tRNAs. Complementary oligonucleotide binding results show, in spite of its unusual biological role, its secondary and tertiary structure are not drastically different from that of other tRNAs. The binding pattern to the anticodon loop is similar to that of other tRNAs. The TψC loop, in spite of the fact it does not contain the sequence T-ψ-C, does not bind its complementary oligomers. The amino acid acceptor end binds its complementary oligomers, although it does so more weakly than many other tRNAs. The dihydrouracil loop of yeast initiator tRNA is unusual; it binds no complementary oligomers. Comparison of these binding results with those for oligomers binding to other tRNAs suggests the overall three-dimensional structure of yeast initiator tRNA is similar to that of other tRNAs. The structure indicated by oligonucleotide binding results is on the whole compatible with the crystal structure of yeast $tRNA^{Phe}$.

Acknowledgments

The authors thank Mr. David Koh and Ms. Barbara Dengler for assistance in oligomer synthesis, Ms. Suzanne Dykstra and Mr. Howard Chew for assistance in the tRNA purification, and Dr. Olke Uhlenbeck and Dr. Paul Sigler for materials and helpful discussions regarding this research.

References

Cameron, V., and Uhlenbeck, O. C. (1973), *Biochem. Biophys. Res. Commun. 50,* 635.

Cerutti, P., Miles, H. T., and Frazier, J. (1966), *Biochem. Biophys. Res. Commun. 22,* 466.

Dube, S. K., Marcker, K. A., Clark B. F. C., and Cory, S. (1968), *Nature (London) 218,* 232.

Eisinger, J., Feuer, B., and Yamane, T. (1971), *Nature (London), New Biol. 231,* 126.

Eisinger, J., and Spahr, P. (1973), *J. Mol. Biol. 73,* 131.

Haselkorn, R., and Rothman-Denes, L. B. (1973), *Ann. Rev. Biochem. 42,* 397.

Högenauer, G. (1970), *Eur. J. Biochem. 12,* 527.

Högenauer, G., Turnowsky, F., and Unger, F. M. (1972), *Biochem. Biophys. Res. Commun. 46,* 2100.

Kim, S. H., Suddath, F. L., Quigley, G. J., McPherson, A., Sussman, J. L., Wang, A. H. J., Seeman, N. C., and Rich, A. (1974a), *Science 185,* 435.

Kim, S. H., Sussman, J. L., Suddath, F. L., Quigley, G. J., McPherson, A., Wang, A. H. J., Seeman, N. C., and Rich, A. (1974b), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4970.

Levine, M. (1974), Ph.D. Thesis, University of California, Berkeley.

Lewis, J. B., Brass, L. F., and Doty, P. (1975), *Biochemistry* (in press).

Miller, P. S., Barret, J. C., and Ts'o, P. O. P. (1974), *Biochemistry 13,* 4887.

Pasek, M., Venkatappa, M. P., and Sigler, P. B. (1973), *Biochemistry 12,* 4834.

Piper, P. W., and Clark, B. F. C. (1973), *FEBS Lett. 30,* 265.

Pongs, O., Bald, R., and Reinwald E. (1973), *Eur. J. Biochem. 32,* 117.

Pongs, O., and Reinwald, E. (1973), *Biochem. Biophys. Res. Commun. 50,* 357.

Pongs, O., Reinwald, E., and Stamp, K. (1971), *FEBS Lett. 16,* 275.

RajBhandary, U. L., and Ghosh, H. P. (1969), *J. Biol. Chem. 244,* 1104.

Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C., and Klug, A. (1974), *Nature (London) 250,* 546.

Schimmel, P. R., Uhlenbeck, O. C., Lewis, J. B., Dickson, L. A., Eldred, E. W., and Schreier, A. A. (1972), *Biochemistry 11,* 642.

Simsek, M., and RajBhandary, U. L. (1972), *Biochem. Biophys. Res. Commun. 49,* 508.

Simsek, M., Ziegenmeyer, J., Heckman, J., and RajBhandary, U. L. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 1041.

Stewart, J. W., Sherman, F., Shipman, N. A., and Jackson, M. (1971), *J. Biol. Chem. 246,* 7429.

Uhlenbeck, O. C. (1972), *J. Mol. Biol. 65,* 25.

Uhlenbeck, O. C., Baller, J., and Doty, P. (1970), *Nature (London) 225,* 508.

Uhlenbeck, O. C., Chirikjian, J. G., and Fresco, J. R. (1974), *J. Mol. Biol. 89,* 495.