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Codon–anticodon interaction at the ribosomal P (peptidyl-tRNA) site

(tRNA binding/aminoacyl-tRNA site/puromycin reaction/tetracycline/viomycin)

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ABSTRACT **A method for binding tRNA to ribosomes, introduced by Watanabe [Watanabe, S. (1972) *J. Mol. Biol.* 67, 443–457], permits nonenzymatic binding of *N*-acetyl-Phe-tRNA^{Phe} to either the ribosomal aminoacyl-tRNA (A) or peptidyl-tRNA (P) site with almost 100% specificity. We used this method to analyze a possible codon–anticodon interaction at the P site for NH_2-blocked aminoacyl-tRNA and deacylated tRNA. *N*-Acetyl-Phe-tRNA^{Phe} bound only to the P site of poly(U)-programmed 70S ribosomes, not to poly(A)-programmed ribosomes. The reverse mRNA dependence was found for *N*-acetyl-Lys-tRNA^{Lys}. A series of purified deacylated tRNAs was analyzed in the poly(U) and poly(A) system for abilities to block P-site binding of *N*-acetyl-aminoacyl-tRNA and to direct the *N*-acetyl-aminoacyl-tRNA to the A site. Only the cognate tRNA was as effective as the bulk tRNA at a concentration of less than 1/20th that of bulk tRNA. tRNAs whose corresponding codons are identical or similar (same base character) in the first two codon positions showed a low but significant effect. The other noncognate tRNAs were unable to direct the NH_2-blocked aminoacyl-tRNAs to the A site. Chlortetracycline interfered neither with the P-site binding of NH_2-blocked aminoacyl-tRNA nor with the effects of deacylated tRNAs. Furthermore, the translocation blocker viomycin affected neither the binding to the A site nor that to the P site. These effects of both antibiotics indicate that both kinds of tRNA do not bind transiently in the A site before filling the P site and that codon–anticodon interaction takes place at the P site.**

At least two tRNA molecules are bound to a ribosome active in protein synthesis. Aminoacyl-tRNA selected by codon–anticodon interaction binds to the A site, whereas the P site is occupied by the peptidyl-tRNA.

Little is known of the fate of the codon–anticodon interaction after translocation to the P site. This is true for both the peptidyl-tRNA and the tRNA deprived of the peptidyl residue but still present in the P site. A possible codon–anticodon interaction at the P site creates an immediate structural problem. The two adjacent codons in the A and P sites are both 1 nm (10 Å) long, but each binds a tRNA molecule with a diameter of 2 nm (20 Å; 1, 2). Therefore, the simplest possibility for the accommodation of two tRNA molecules would be the absence of a codon–anticodon interaction at the P site.

The initiation-specific fMet-tRNA$_f^{Met}$ is a peptidyl-tRNA analogue and binds codon-dependently to the P site (3). However, due to its many specific features, the binding of fMet-tRNA$_f^{Met}$ does not seem to be an appropriate model for the P-site binding during the elongation cycle. For example, binding of the initiator tRNA requires a specific factor (IF-2; for review see ref. 4). This tRNA species can be bound to 23S RNA, forming a stretch of 17 intermolecular base pairs (5). A unique interaction of mRNA and 16S RNA exists during the initiation process (6, 7). Furthermore, it is doubtful whether the binding of tRNAs to oligonucleotide-programmed ribosomes properly reflects the codon–anticodon relationship on the ribosome in presence of mRNA.

Deacylated tRNA was claimed to bind codon-dependently to the P site as deduced from the stimulation of the binding of labeled homopolynucleotides to ribosomes by the cognate tRNAs (8). However, the authors did not determine the binding site of the effective tRNA and it cannot be excluded that the stimulation of mRNA binding was due to cognate deacylated tRNA present in the A site. In addition to the well documented P-site binding (9–11), deacylated tRNA can also bind to the A site—e.g., as a trigger of the stringent response (12, 13). The latter binding to the A site is codon dependent. However, no information is available whether the deacylated tRNA bound to the P site undergoes codon–anticodon interaction or not.

Recently, we demonstrated that deacylated tRNA binds preferentially to the P-site region of isolated 50S subunits—i.e., in the absence of 30S subunits and mRNA (14). This finding points to the possibility that at least the deacylated tRNA could be bound to the P site in 70S ribosomes without codon–anticodon interaction.

In order to test this possibility we analyzed the binding of both *N*-acetyl-aminoacyl-tRNA (a peptidyl-tRNA analogue) and deacylated tRNA to the P site. In this paper we demonstrate that the binding of both tRNA species to the P site depends on codon–anticodon interaction.

MATERIALS AND METHODS

Ribosomes, tRNAs, and Amino Acids. Ribosomes and their subunits were isolated from *Escherichia coli* K-12 cells (strain A19) as described (15). Tight-coupled 70S ribosomes were a kind gift from R. Lührmann (Max-Planck Institut für Molekulare Genetik, Berlin). tRNA was purchased from Boehringer Mannheim, West Germany [bulk tRNA from *E. coli*, tRNA^{Phe} (1263 pmol/A_{260} unit), tRNA^{Lys} (980 pmol/A_{260} unit), tRNA^{Glu} (1126 pmol/A_{260} unit), tRNA^{Gly} (900 pmol/A_{260} unit)]; Sigma [tRNA^{Tyr} (1070 pmol/A_{260} unit)]; and Miles [tRNA^{Arg} (1220 pmol/A_{260} unit), tRNA^{Leu} (1400 pmol/A_{260} unit)].

[^{14}C]Phenylalanine (specific activity 19.3 GBq/mmol = 552 mCi/mmol) and [^{3}H]lysine (specific activity 925 GBq/mmol = 25 Ci/mmol) were obtained from Amersham (England). 7-Chlortetracycline was from Serva (Heidelberg, West Germany), and viomycin was from Pfizer (Tokyo). Preparation of elongation factor G (EF-G) followed ref. 16.

The tRNAs were charged as described (16), with the exception that the S-150 enzymes were freed of the tRNA on a Whatman DE-52 DEAE-cellulose column. *N*-Acetylation of the tRNAs followed ref. 17.

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Abbreviations: A site, aminoacyl-tRNA site; P site, peptidyl-tRNA site; Ac-Phe-tRNA^{Phe}, *N*-acetyl-Phe-tRNA^{Phe}; EF, elongation factor.

Binding of tRNA to Ribosomes and Translocation. Nonenzymatic binding of tRNA to ribosomes was a modification of the method of Watanabe (18). The procedure was performed in three steps. (*i*) *Preincubation of 70S ribosomes, poly(U), and, where indicated, deacylated tRNA.* A 300-μl reaction mixture containing 50 mM Tris·HCl at pH 7.8, 10 mM Mg acetate, 160 mM NH_4Cl, 5 mM 2-mercaptoethanol, 75 μg of poly(U), 20–24 A_{260} units of 70S ribosomes (usually 12 A_{260} units of 30S and 16 units of 50S subunits), and, where indicated, 3 A_{260} units of bulk tRNA or 0.15 A_{260} unit of specific purified tRNA was incubated for 10 min at 37°C. (*ii*) *Ac-[^{14}C]Phe-tRNA^{Phe} binding.* The volume of the preincubation mixture was increased to 600 μl upon addition of 40 pmol of Ac-[^{14}C]-Phe-tRNA^{Phe}. The final concentrations were 50 mM Tris·HCl at pH 7.8, 15 mM Mg acetate, 160 mM NH_4Cl, and 5 mM 2-mercaptoethanol. After an incubation of 30 min at 37°C, two 100-μl aliquots were removed and each was diluted with 2 ml of binding buffer (50 mM Tris·HCl, pH 7.5/15 mM Mg acetate/160 mM NH_4Cl/5 mM 2-mercaptoethanol). The solutions were filtered (nitrocellulose filter; Sartorius, Göttingen, West Germany; catalog no. 11306), and the filters were rinsed two times with 2 ml of binding buffer and dried under an infrared lamp, and radioactivity was measured. (*iii*) *Puromycin reaction without and with EF-G.* The remaining 400-μl binding mixture received 60 μl of binding buffer containing 0.6 mM GTP, 6 mM phospho*enol*pyruvate, and 4 μg of pyruvate kinase. Four aliquots of 100 μl each were removed and mixed with 10 μl of buffer [10 mM Tris·HCl, pH 7.8/10 mM Mg acetate/100 mM KCl/10 mM 2-mercaptoethanol/20% (vol/vol) glycerol containing, when indicated, 2 μg of EF-G]. The solutions were incubated for 10 min at 37°C. Ten microliters of a 10 mM puromycin solution in binding buffer was added to each aliquot, and the mixture was incubated for 30 min at 0°C. The reaction was stopped with 100 μl of 0.3 M Na acetate, pH 5.5, in saturated $MgSO_4$. Ethyl acetate (1.5 ml) was added and, after vigorous shaking (1 min) and phase separation (5 min, 0°C), the upper 1 ml was removed and assayed for radioactivity.

When poly(A)-directed tRNA binding was measured, the same experimental scheme was followed with the exception that poly(U) and Ac-[^{14}C]Phe-tRNA^{Phe} were replaced by the same amounts of poly(A) and Ac-[^{3}H]Lys-tRNA^{Lys}, respectively. Only half of the amounts of deacylated tRNAs were used. Because Ac-Lys-puromycin is poorly extracted in the peptidyltransferase assay, [^{3}H]Lys with a high specific activity was used. All assays described in the following experiments have been performed in duplicate.

RESULTS

Specificity of Nonenzymatic Binding of Ac-Phe-tRNA^{Phe} to Either A or P Site. Table 1, experiment 1, demonstrates that Ac-Phe-tRNA^{Phe} can be bound nonenzymatically with a striking specificity to either the P site or the A site, in agreement with a previous report (18). Without deacylated tRNA, the same amount of Ac-Phe-puromycin was formed irrespective of the presence of EF-G (see first line in Table 1, experiment 1). This means that the Ac-Phe-tRNA^{Phe} was already present in the P site and must not be translocated from the A site. However, when deacylated tRNA was present, the puromycin reaction required the presence of EF-G. This observation indicates that Ac-Phe-tRNA^{Phe} was present in the A site and must be translocated to the P site in order to become puromycin sensitive. Obviously, the deacylated tRNA occupies the P site, thus directing the Ac-Phe-tRNA^{Phe} to the A site. This effect of the deacylated tRNA is our operational definition for a P-site-bound deacylated tRNA. The fact that chlortetracycline (50 μM) drastically inhibited the binding to the A site but not that to the P site is further indicative of the specificity of binding.

When mRNA was omitted, low but significant binding of Ac-Phe-tRNA^{Phe} was found (Table 1, experiment 2). Both the positive puromycin reaction and the finding that preincubation with deacyl-tRNA abolished the binding indicate that the NH_2-blocked aminoacyl-tRNA binds to the P site of nonprogrammed ribosomes.

Analysis of the Binding of Peptidyl-tRNA Analogues. Efficient binding of peptidyl-tRNA analogues to the P site depends on the presence of mRNA (see, for example, ref. 18).

Table 1. Specificity of the binding of Ac-[^{14}C]Phe-tRNA^{Phe} to the ribosomal A and P sites

Exp.	Poly(U)	Preincubation with deacylated tRNA	Chlortetracycline	Binding of Ac-Phe-tRNA^{Phe}, cpm	Ac-Phe-puromycin formed, cpm: Without translocation (−EF-G)	Ac-Phe-puromycin formed, cpm: With translocation (+EF-G)	Remarks
1	+	−	−	6440	3400	3390	100% P-site binding
	+	+	−	4530	35	1910	98% A-site binding
	+	−	+	6380	3420	3420	No inhibition of P-site binding
	+	+	+	1010	70	630	78% inhibition of A-site binding
			Control: minus 70S	210	350	360	
2	−	−	−	630	830	750	
	−	+	−	20	20	30	
	+	−	−	4910	2210	2400	
	+	+	−	2290	50	1290	
			Control: minus 70S	260	480	470	

The background values "minus 70S" have been subtracted. Deacylated tRNA, bulk tRNA from *E. coli*. Chlortetracycline (50 μM) was added after preincubation. Exp. 1 was performed with 70S ribosomes (tight couples, ref. 19) and at the same time with equivalent amounts of (30S + 50S) subunits (data not shown). The experiment with the subunits gave qualitatively the same results; the P-site binding (minus deacyl-tRNA) was reduced to 60% of the above values, whereas the same A-site binding was found. Exp. 2 was performed with (30S + 50S) subunits; a similar experiment with poly(A) and Ac-Lys-tRNA^{Lys} gave the equivalent results except that relatively smaller (but still significant) amounts of Ac-Lys-puromycin were found in the case of minus poly(A) and minus deacylated tRNA. In the presence of mRNA the binding data were always higher than those obtained in the puromycin reaction. This difference is due to the different preparation methods. For the determination of the binding data the total aliquot was applied to the filter, whereas during the preparation for the puromycin reaction and the ethyl acetate extraction, about 45% of the material was discarded. Therefore, the binding data should not be compared directly with the puromycin data.

Table 2. Binding of peptidyl-tRNA analogues directed by poly(U) or poly(A)

mRNA	tRNA	Peptidyl-tRNA analogue binding: Site	Species	Codon specificity	cpm
Poly(U)	−	P	Ac-Phe-	Cognate	4530
	+	A			4200
	−	P	Ac-Lys-	Non-	1170
	+	A	$tRNA^{Lys}$	cognate	190
Poly(A)	−	P	Ac-Lys-	Cognate	8200
	+	A	$tRNA^{Lys}$		8320
	−	P	Ac-Phe-	Non-	620
	+	A	$tRNA^{Phe}$	cognate	170

The background values (minus 70S), 190 cpm and 410 cpm for Ac-Phe-$tRNA^{Phe}$ and Ac-Lys-$tRNA^{Lys}$, respectively, have been subtracted. In this experiment 30S and 50S subunits were used.

Table 2 demonstrates that this effect is not an unspecific codon-independent stimulation of the P-site binding, but the stimulatory effect depends on the codon present in the P site. The cognate *N*-acetyl-aminoacyl-tRNAs [Ac-Phe-$tRNA^{Phe}$ for poly(U) and Ac-Lys-$tRNA^{Lys}$ for poly(A)] bound equally well to both A and P sites. The noncognate tRNAs did not bind to the ribosomal A site and showed low binding (about 15%) to the P site. Nevertheless, more than 85% of the *N*-acetyl-aminoacyl-tRNA bound codon-specifically to the P site.

Analysis of the Binding of Deacylated tRNA. During the elongation cycle the tRNA can exist in two different states while in the P site, either as a peptidyl-tRNA after translocation or as deacylated tRNA after peptidyl transfer. According to the operational definition outlined above, the deacylated tRNA is bound at the P site when it prevents the binding of peptidyl-tRNA to the P site but directs the peptidyl-tRNA to the A site. In the following experiment we tested whether this tRNA effect depends on the codon present in the P site.

We collected a series of purified tRNAs and ordered them according to the similarity of their respective codons to the Phe codon UUU (top of Table 3) and to the Lys codon AAA (bottom of Table 3). The puromycin reaction was measured (*a*) without and (*b*) with concomitant translocation. The *b*/*a* ratio appears to be a sensitive measure for the deacylated tRNA effect. A ratio of 0.8–1.2 means that the translocation reaction did not significantly increase the puromycin reaction; i.e., at least 85% of the peptidyl-tRNA analogue bound to the P site. In contrast, ratios >1.2 indicate significant A-site binding.

Table 3 shows that without deacylated tRNA all the Ac-Phe-$tRNA^{Phe}$ bound to the P site (*b*/*a* ratio 1.0), whereas the addition of the optimal amount of the bulk tRNA [3 A_{260} units per poly(U) assay] forced the peptidyl-tRNA to bind to the A site (*b*/*a* = 16.7). About the same effect could be achieved with 1/20th the amount of purified $tRNA^{Phe}$ (*b*/*a* = 18.1). $tRNA^{Leu}$, with a corresponding codon most similar to UUU, was found to influence the Ac-Phe-$tRNA^{Phe}$ binding to a low but significant extent (*b*/*a* = 1.4) that was more pronounced at the higher tRNA concentration. The other tRNAs showed either a questionable effect ($tRNA^{Tyr}$, $tRNA^{Arg}$, $tRNA^{Gly}$) or definitely no effect ($tRNA^{Glu}$, $tRNA^{Lys}$) even at the high tRNA concentration.

$tRNA^{Phe}$ at high concentrations could reduce the Ac-Phe-$tRNA^{Phe}$ binding to the A site (see Table 3 at 0.6 A_{260} unit of $tRNA^{Phe}$). This effect was analyzed in more detail, and the results are presented in Fig. 1. At low amounts of $tRNA^{Phe}$ (0.06 A_{260} unit) the total binding of Ac-Phe-$tRNA^{Phe}$ was not much influenced (75% binding), whereas the puromycin reaction

Table 3. Analysis of the binding of deacylated tRNA to the ribosome

Deacylated tRNA present during the preincubation: Species	Corresponding codons	A_{260} units per poly(U) assay	Poly(U)-directed Ac-Phe-puromycin formation: −EF-G (*a*), cpm	+EF-G (*b*), cpm	*b*/*a*	Poly(A)-directed Ac-Lys-puromycin formation: −EF-G (*a*), cpm	+EF-G (*b*), cpm	*b*/*a*
—	—	—	1120	1130	1.0	1986	2068	1.0
$tRNA_{E.\ coli}$		0.6	430	1241	2.9	870	2570	3.0
		3.0	60	1002	16.7	129	890	6.9
$tRNA^{Phe}$	UUU (C)	0.15	60	1086	18.1	879	729	0.8
		0.6	14	760	54.3	382	310	0.8
$tRNA^{Leu}$	CUU (C, A, G),	0.15	644	875	1.4	957	1085	1.1
	UUA (G)	0.6	395	1097	2.8	411	333	0.8
$tRNA^{Tyr}$	UAU (C)	0.15	846	912	1.1	1213	1366	1.1
		0.6	975	1145	1.2	724	624	0.9
$tRNA^{Arg}$	CGU (C, A, G)	0.15	781	792	1.0	1567	1654	1.1
		0.6	941	1121	1.2	892	878	1.0
$tRNA^{Gly}$	GGU (C, A, G)	0.15	571	633	1.1	1767	2300	1.3
		0.6	491	582	1.2	894	2484	2.8
$tRNA^{Glu}$	GAA (G)	0.15	728	650	0.9	1497	1734	1.2
		0.6	479	411	0.9	991	1592	1.6
$tRNA^{Lys}$	AAA (G)	0.15	850	833	1.0	411	2311	5.6
		0.6	629	683	1.1	51	827	16.2

In the poly(U)-directed system, 70S ribosomes, poly(U), and the indicated deacylated tRNA were preincubated prior to addition of Ac-Phe-$tRNA^{Phe}$. The puromycin reaction was performed before and after translocation (−EF-G and +EF-G, respectively). The poly(A)-directed formation of *N*-acetyl-lysyl-puromycin was determined similarly, with the exception that half of the amount of deacylated tRNA indicated for the poly(U) system (third column) was used. The low concentration of deacylated tRNA corresponds to about one deacylated tRNA molecule per 70S ribosome in the poly(A) system and to about two in the poly(U) system. The designation CUU (C, A, G) for the leucine codon, for example, indicates that the third base can be C, A, or G instead of U. For arginine we omitted the possible codons AGA and AGG, which are similar to AAA for lysine, because the $tRNA^{Arg}$ used did not behave like a tRNA with an anticodon similar to $tRNA^{Lys}$, in contrast to the behavior of $tRNA^{Gly}$ or $tRNA^{Glu}$ [see poly(A)-directed puromycin reaction].

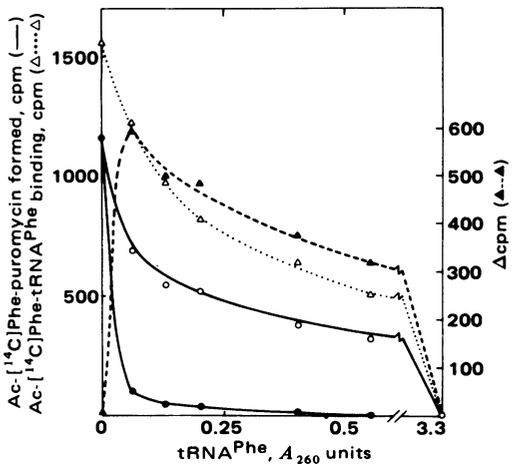

FIG. 1. Effects of various amounts of deacylated $tRNA^{Phe}$ on the ribosomal binding of Ac-Phe-$tRNA^{Phe}$. The binding to poly(U)-programmed 70S ribosomes was tested (Δ·····Δ). The formation of *N*-acetyl-Phe-puromycin was determined without (P site, ●—●) and with translocation (A and P sites, ○—○). The difference of the latter two (Δcpm, ▲---▲) gives the amount of Ac-Phe-$tRNA^{Phe}$ specifically bound to the A site.

showed the expected specificity—i.e., more than 80% of the Ac-Phe-tRNA was present in the A site. However, when higher $tRNA^{Phe}$ amounts were added, both total binding and A-site binding were reduced and even abolished in the presence of 3.3 A_{260} units of $tRNA^{Phe}$ (compare the curve for total binding with the difference curve). Thus, after occupying the P site the cognate deacylated $tRNA^{Phe}$ binds also to the A site, causing the decrease of Ac-Phe-$tRNA^{Phe}$ binding to the A site.

Results equivalent to those obtained in the presence of poly(U) were seen in the poly(A) system (Table 3). The cognate deacylated tRNA ($tRNA^{Lys}$) caused a striking binding of Ac-Lys-$tRNA^{Lys}$ to the A site. $tRNA^{Gly}$ and $tRNA^{Glu}$, whose corresponding codons most closely resemble the codon of $tRNA^{Lys}$, only slightly influenced the binding pattern of the peptidyl-tRNA analogue ($b/a > 1.2$). This binding pattern was not at all affected by the remaining tRNAs tested. However, at very high concentrations these tRNAs could reduce the total binding of the Ac-Lys-$tRNA^{Lys}$ (data not shown) as indicated by the reduced puromycin reaction [compare $tRNA^{Leu}$ at the low and high concentrations in the poly(A) system]. Obviously, those noncognate tRNAs that cannot form a codon–anticodon interaction somehow interacted with the P site, which likely results in a slow-down of binding for the peptidyl-tRNA analogue. However, these tRNAs never triggered peptidyl-tRNA binding to the A site. The latter effect is a specific feature of those tRNAs that can undergo a codon–anticodon interaction at the P site.

A possible explanation of the preceding results could be that the deacylated tRNA binds via a transient A-site binding to the P site, thus mimicking a codon requirement for the P site. Chlortetracycline and viomycin were used to test this hypothesis. The antibiotic viomycin stabilizes 70S couples (20) and specifically blocks the translocation reaction (21, 22). Poly(U)-programmed 70S ribosomes were preincubated with $tRNA^{Phe}$ in the presence or absence of the drug(s). The complex was pelleted to remove the unbound $tRNA^{Phe}$, resuspended in the ionic milieu of the preincubation, and tested for $tRNA^{Phe}$ present in the P site.

In this experiment, chlortetracycline reduced to some extent the binding of Ac-Phe-tRNA to the P site (Table 4, compare lines 1 and 3). Likewise, the binding of deacylated tRNA to the P site was inhibited to a small extent as indicated by the small

Table 4. Poly(U)-dependent binding of $tRNA^{Phe}$ in the presence of chlortetracycline and viomycin

	Preincubation		Binding of Ac-Phe-$tRNA^{Phe}$, cpm	Ac-Phe-puromycin, cpm	
	$tRNA^{Phe}$	Drug		−EF-G (P site)	+EF-G (A and P sites)
1	−	—	3410	1620	1770
2	+	—	2440	110	1495
3	−	CTET	2380	1885	1855
4	+	CTET	870	370	915
5	−	VIO	3400	920	990
6	+	VIO	2120	460	485
7	−	CTET + VIO	2640	1360	1510
8	+	CTET + VIO	970	360	400

The background values (minus 70S ribosomes) have been subtracted (binding, 93 cpm; −EF-G, 303 cpm; +EF-G, 286 cpm). The preincubation was performed as described in *Materials and Methods*, except that 50 μM chlortetracycline (CTET) and 40 μM viomycin (VIO) were present during the whole procedure when indicated. After preincubation 100 μl of 40% (wt/vol) polyethyleneglycol-6000 in a buffer containing 50 mM Tris·HCl at pH 7.8, 10 mM Mg acetate, 100 mM NH_4Cl, and 5 mM 2-mercaptoethanol was added to the 300-μl preincubation mixture, which was kept for 10 min at 0°C and centrifuged for 10 min at 30,000 × *g*. The pellet was resuspended in 300 μl of the same buffer and the assay was continued as described in *Materials and Methods*.

amount of Ac-Phe-tRNA found in the P site (compare lines 4 and 2). These effects of chlortetracycline on the P-site binding were not seen when the pelleting step was omitted (see Table 1, experiment 1). Thus, in the presence of chlortetracycline a small amount of tRNA was removed from the P site during the pelleting step. Alternatively, if the tRNA bound to the P site via a transient A-site binding, chlortetracylcine could weaken the transient A-site binding, thus preventing the filling of the P site by tRNA. Although this alternative was not supported by the binding experiment without a pelleting step (see Table 1, experiment 1) this possibility was tested in more detail by use of the antibiotic viomycin.

Viomycin did not influence the P-site binding of Ac-Phe-tRNA (compare lines 5 and 1, in Table 4), but it blocked the translocation reaction (see line 6) in accordance with the effects reported (21, 20). Viomycin inhibited the puromycin reaction (compare lines 5 and 1), in contrast to a previous report (22). However, this effect was less pronounced than the blockage of the translocation, and was not seen when the peptidyltransferase activity was measured in the fragment assay in the presence of 33% ethanol (data not shown). When both antibiotics were present the inhibition patterns of both drugs were expressed (lines 7 and 8): the binding to the A site was reduced (chlortetracycline effect: 970 cpm in line 8) and the translocation was blocked (viomycin effect: 360 and 400 cpm in line 8). However, although the translocation reaction was blocked completely, the same small amount of deacylated tRNA bound to the P site as in the case in which chlortetracyline only was present (360 and 370 cpm in lines 8 and 4, respectively).

In summary, viomycin did not affect either A- or P-site binding at concentrations at which translocation was blocked completely. Chlortetracycline, severely inhibiting the A-site binding, scarcely reduced the P-site binding. The latter effect was not influenced by the additional presence of viomycin. Clearly, a transient A-site binding is not an essential prerequisite for a codon-dependent P-site binding, and the codon dependence of the P-site binding is not caused by a codon-dependent interaction of the A site preceding a translocation of the tRNA to the P site.

We conclude that cognate deacylated tRNA binds directly to the P site and that its anticodon is hydrogen-bonded to the codon present in the P site.

DISCUSSION

A method for nonenzymatically binding tRNAs to ribosomes, introduced by Watanabe (18), permits a highly specific binding of Ac-Phe-tRNA^{Phe} to either the A or the P site (>95% and 100% specificity, respectively, see Table 1). An important advantage of this method is the fact that the binding of NH_2-blocked aminoacyl-tRNA to either the A or the P site is performed at the same Mg^{2+} concentration (15 mM), which excludes Mg^{2+}-induced changes of tRNA or ribosome structure.

Both deacylated tRNA and NH_2-blocked aminoacyl-tRNA bind first to the P site, and only when this site is occupied do they bind to the A site (Fig. 1 and ref. 18, respectively) as was reported by Lapidot's group (23, 24).

The preferential binding of both kinds of tRNA to the P site can be observed even with isolated 50S subunits in the presence of alcohol (refs. 14 and 25, respectively). Furthermore, 70S ribosomes without mRNA bind low amounts of peptidyl-tRNA analogues (Table 1, experiment 2). These peptidyl-tRNA analogues are present exclusively in the P site (Table 1). Thus, both deacylated tRNA and NH_2-blocked aminoacyl-tRNA bind preferentially to the P site of 50S subunits (in the presence of alcohol), of nonprogrammed 70S ribosomes and, with increased efficiency, to that of programmed 70S ribosomes.

In this paper we used the nonenzymatic binding system to study codon–anticodon interactions in the P site. Our analysis revealed that the binding of both deacylated and NH_2-blocked aminoacyl-tRNA to the P site of programmed 70S ribosomes depends on the presence of the appropriate codon (Tables 3 and 2, respectively). Chlortetracycline severely inhibits the binding of the peptidyl-tRNA analogues to the A site but does not influence the P-site binding of peptidyl-tRNA analogues (refs. 26, 27; see also Table 1, experiment 1) or deacylated tRNA (Table 4). Furthermore, viomycin blocking translocation affects neither the P-site binding of Ac-Phe-tRNA (ref. 21 and Table 4) nor that of deacylated tRNA (Table 4). These findings indicate that both kinds of tRNA bind directly to the P site and that the codon dependence is not mimicked by a transient A-site binding. Further support for a direct access to the P site comes from recent binding experiments with ribosomes that were programmed with a hexanucleotide—e.g., G-U-A-U-U-U. Efficient binding of the ternary complex EF-Tu·Phe-tRNA^{Phe}·GTP was observed only in the presence of tRNA^{Val}, which corresponds to the codon GUA (R. Lührmann, H. Eckhardt, and G. Stöffler, personal communication). If there were no codon–anticodon interaction in the P site and the codon dependence were mimicked by a transient A-site binding, a tRNA^{Phe} rather than a tRNA^{Val} would be required for the binding of the ternary complex.

Preincubation with deacylated tRNA does not lead to A-site binding of NH_2-blocked aminoacyl-tRNA in the case of nonprogrammed ribosomes, in contrast to programmed ribosomes (Table 1, experiment 2). This finding, together with the effects of the cognate deacyl-tRNA in the P site (Table 3), strikingly indicates that codon–anticodon interaction in the P site is a prerequisite for tRNA binding to the A site. This requirement for the A-site binding explains the successive occupation of the ribosomal sites in the order P site → A site. Obviously, for this reason the initiator tRNA fMet-tRNA$_f^{Met}$ binds directly to the P site during the initiation process.

It appears that the codon–anticodon interaction persists during several steps comprising about one and a half rounds of the elongation cycle, starting with the codon-directed binding of the aminoacyl-tRNA to the A site and ending with the deacylated tRNA bound in the P site. Therefore, we are facing the problem that two tRNAs—i.e., the peptidyl-tRNA (P site) and the aminoacyl-tRNA (A site)—each 2 nm (20 Å) in diameter bind with their anticodons to adjacent codons each 1 nm (10 Å) in length. Furthermore, the tRNA 3′ ends carrying the peptidyl-residue and aminoacyl-residue, respectively, must be side by side in order to allow for peptidyl transfer. These requirements can be fulfilled by the L-shaped tRNA (28), and, probably, they are the reasons why tRNAs have evolved to this tertiary structure.

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1. Suddath, F. L., Quigley, G. J., McPherson, A., Sneden, D., Kim, J. J., Kim, S. H. & Rich, A. (1974) *Nature (London)* **248,** 20–24.
2. Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C. & Klug, A. (1974) *Nature (London)* **250,** 546–551.
3. Kuechler, E. & Rich, A. (1970) *Nature (London)* **225,** 920–924.
4. Grunberg-Manago, M. & Gros, F. (1977) in *Progress in Nucleic Acid Research and Molecular Biology*, ed. Cohn, W. E. (Academic, New York), Vol. 20, pp. 209–284.
5. Dahlberg, J. E., Kintner, G. & Lund, E. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1071–1075.
6. Shine, J. & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1342–1346.
7. Steitz, J. A. & Jakes, K. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4734–4738.
8. Grasmuk, H., Nolan, R. D. & Drews, J. (1975) *FEBS Lett.* **53,** 229–233.
9. Leder, P. & Bursztyn, H. (1966) *Proc. Natl. Acad. Sci. USA* **56,** 1579–1585.
10. Zasloff, M. (1973) *J. Mol. Biol.* **76,** 445–453.
11. Kyner, D., Zabes, P. & Levin, D. H. (1973) *Biochim. Biophys. Acta* **324,** 386–396.
12. Pedersen, F. S., Lund, E. & Kjeldgaard, N. O. (1973) *Nature (London) New Biol.* **243,** 13–15.
13. Haseltine, W. W. & Block, R. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1564–1568.
14. Ulbrich, B., Mertens, G. & Nierhaus, K. H. (1978) *Arch. Biochem. Biophys.* **190,** 149–154.
15. Schreiner, G. & Nierhaus, K. H. (1973) *J. Mol. Biol.* **81,** 71–82.
16. Wurmbach, P. & Nierhaus, K. H. (1979) *Methods Enzymol.* **60,** 593–606.
17. Haenni, A.-L. & Chapeville, F. (1966) *Biochim. Biophys. Acta* **114,** 135–148.
18. Watanabe, S. (1972) *J. Mol. Biol.* **67,** 443–457.
19. Hapke, B. & Noll, H. (1976) *J. Mol. Biol.* **105,** 97–109.
20. Yamada, T. & Nierhaus, K. H. (1978) *Mol. Gen. Genet.* **161,** 261–265.
21. Lion, Y. F. & Tanaka, N. (1976) *Biochem. Biophys. Res. Commun.* **71,** 477–483.
22. Modolell, J. & Vazquez, D. (1977) *Eur. J. Biochem.* **81,** 491–497.
23. de Groot, N., Panet, A. & Lapidot, Y. (1971) *Eur. J. Biochem.* **23,** 523–527.
24. Hamburger, A. D., Lapidot, Y. & de Groot, N. (1973) *Eur. J. Biochem.* **32,** 576–583.
25. Monro, R. E., Černá, J. & Marcker, K. A. (1968) *Proc. Natl. Acad. Sci. USA* **61,** 1042–1049.
26. Suarez, G. & Nathans, D. (1965) *Biochem. Biophys. Res. Commun.* **18,** 743–750.
27. Bodley, J. W. & Zieve, F. J. (1969) *Biochem. Biophys. Res. Commun.* **36,** 463–468.
28. Rich, A. (1974) in *Ribosomes*, eds. Nomura, M., Tissières, A. & Lengyel, P. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 871–884.