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Properties of tRNA Species Modified in the 3′-Terminal Ribose Moiety in an Eukaryotic Ribosomal System†

E. Baksht, N. de Groot, M. Sprinzl,* and F. Cramer

ABSTRACT: Phe-tRNA^{Phe} species modified on the 3′-terminal ribose residue were investigated for their ability to participate in individual steps of the elongation cycle using eukaryotic ribosomes from reticulocytes. None of the Phe-tRNAs used, namely Phe-tRNA^{Phe}-C-C-3′dA, Phe-tRNA^{Phe}-C-C-3′-NH_2A, and Phe-tRNA^{Phe}-C-C-$A_{oxi-red}$, can function in the overall process. All modified Phe-tRNA^{Phe} species can be bound nonenzymatically to ribosomes. Phe-tRNA^{Phe}-C-C-3′NH_2A exhibits exceptionally high binding at low Mg^{2+} concentration compared with Phe-tRNA^{Phe}-C-C-A binding. Ac-Phe-tRNA^{Phe} species prepared from the three modified tRNAs, when bound to the donor site, were devoid of donor activity. The enzymatic binding of both Phe-tRNA^{Phe}-C-C-3′dA and Phe-tRNA^{Phe}-C-C-3′NH_2A is less efficient than that of Phe-tRNA^{Phe}-C-C-A but these Phe-tRNA^{Phe} species have acceptor activity. Phe-tRNA^{Phe}-C-C-$A_{oxi-red}$ is not a substrate for the EF-I promoted binding reaction and has no acceptor activity. The nonaminoacylated species, tRNA^{Phe}-C-C-A, tRNA^{Phe}-C-C-3′dA, and tRNA^{Phe}-C-C-3′NH_2A, bind to the ribosome to a larger extent than the corresponding aminoacylated tRNAs, both in the presence and in the absence of poly(U). Peptidyl-tRNA^{Phe}-C-C-3′dA bound to the donor site cannot activate the acceptor site for EF-I promoted binding of Phe-tRNA^{Phe} as does peptidyl-tRNA^{Phe}-C-C-A. Further, it was observed that a correct codon–anticodon interaction influences the recognition of the 3′ terminus of tRNA. Specificity of eukaryotic ribosomes for the 2′- and/or 3′-aminoacylated tRNA species is discussed and compared with the properties of *Escherichia coli* system.

The binding of tRNA[1] to the ribosome is a multiple-site interaction in which at least three partial sequences of the tRNA seem to be involved: the anticodon, the GpTpΨpC sequence, and the 3′-terminal -C-C-A sequence. It is generally accepted that ribosomes active in protein synthesis bind two molecules of tRNA (Leder, 1973). The 3′-terminal nucleotides of both tRNAs interact with the peptidyl transferase on the large ribosomal subunit. This interaction has been studied in detail, mostly with ribosomes from prokaryotes and it was found that the specificity of the acceptor site (A-site) interaction is different from that of the donor site (P-site) interaction in several respects. A complete -C-C-A terminus bearing the peptidyl residue is required for the binding at the P-site (Monro et al., 1968), but a terminal adenosine seems to be sufficient to replace the aminoacyl-tRNA on the A-site.

tRNA with a modified 3′-terminal ribose moiety can be prepared either by a direct chemical treatment with periodate which reacts in a specific way with the 3′-terminal ribose of the tRNA (Cramer et al., 1968), or by incorporating a modified AMP into tRNA-C-C with tRNA nucleotidyl transferase (Sprinzl et al., 1973b). The modified tRNAs used in this work were tRNA^{Phe}-C-C-3′dA, tRNA^{Phe}-C-C-3′NH_2A, tRNA^{Phe}-C-C-$A_{oxi-red}$, tRNA^{Phe}-C-C-2′dA, and tRNA^{Phe}-C-C (Figure 1). The first three of these tRNAs can be aminoacylated (Sprinzl and Cramer, 1973; Fraser and Rich, 1973) yielding Phe-tRNA^{Phe} species on which the migration of the amino acid residue between the 2′ and the 3′ position of the terminal adenosine (Griffin et al., 1966) cannot take place. These nonisomerizable aminoacyl-tRNAs are therefore suitable for the investigation of the specificity of the tRNA–ribosome interaction with respect to the position of the aminoacyl residue bound to tRNA. The activities of these Phe-tRNA^{Phe} species in a ribosomal system derived from *Escherichia coli* have been studied in several laboratories (Ofengand and Chen, 1972; Chinali et al., 1974; Sprinzl et al., 1975; Hecht et al., 1974; Fraser and Rich, 1973). Some disagreement exists, however, concerning the acceptor activities of the above mentioned, nonisomerizable tRNA species. Chinali et al. (1974) reported that Phe-tRNA^{Phe}-C-C-3′dA and Phe-tRNA^{Phe}-C-C-$A_{oxi-red}$, having the aminoacyl residue on the 2′ position, possess some acceptor activity, while Hecht et al. (1974) could not detect acceptor activity for Phe-tRNA^{Phe}-C-C-3′dA and Hussain and Ofengand (1973) could not observe acceptor activity for enzymatically aminoacylated adenosine$_{oxi-red}$. On the other hand an agreement exists that none

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[1] Abbreviations used are: EF-I, EF-II, elongation factors; tRNA^{Phe}-C-C-A, native phenylalanine transfer ribonucleic acid; Phe-tRNA^{Phe}, phenylalanyl-tRNA^{Phe}; Ac-Phe-tRNA^{Phe}, *N*-acetylphenylalanyl-tRNA^{Phe}; tRNA^{Phe}-C-C, tRNA^{Phe} lacking the 3′-terminal AMP; tRNA^{Phe}-C-C-2′dA or tRNA^{Phe}-C-C-3′dA, tRNA^{Phe} lacking the 2′- or 3′-hydroxyl group of the terminal ribose, prepared by enzymatic incorporation of 2′-deoxyadenosine or 3′-deoxyadenosine 5′-phosphate into tRNA^{Phe}-C-C; tRNA^{Phe}-C-C-3′NH_2A, tRNA^{Phe} with the terminal adenosine replaced by 3′-deoxy-3′-aminoadenosine; tRNA^{Phe}-C-C-$A_{oxi-red}$, tRNA^{Phe} lacking the C(2′)–C(3′) bond of the terminal ribose, prepared by periodate oxidation and subsequent borohydride reduction of tRNA^{Phe}-C-C-A; poly(U), poly(uridylic acid); Tris, tris(hydroxymethyl)aminomethane.

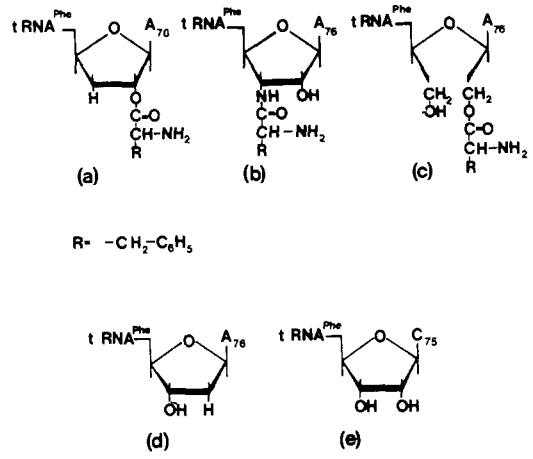

FIGURE 1: Chemical structure of the 3′ terminus of $tRNA^{Phe}$ species: Phe-$tRNA^{Phe}$-C-C-3′dA (a), Phe-$tRNA^{Phe}$-C-C-3′NH_2A (b), Phe-$tRNA^{Phe}$-C-C-$A_{oxi-red}$ (c), $tRNA^{Phe}$-C-C-2′dA (d), and $tRNA^{Phe}$-C-C (e).

of the Ac-Phe-$tRNA^{Phe}$ species derived from these modified tRNA species can function as donor substrates in the peptidyl transferase reaction.

The above mentioned studies have been carried out with prokaryotic ribosomes (70 S), which differ greatly from eukaryotic ribosomes (80 S). The latter are composed of more than 70 different proteins as compared with 55 proteins in the 70S ribosomes and their particle weight is nearly double that of the prokaryotic ribosomes. There also seem to exist differences in the ribosome–tRNA interaction as 80S ribosomes bind nonaminoacylated tRNA with a much greater affinity than the 70S ribosomes (Zasloff, 1973; Kyner et al., 1973). Eckermann et al. (1974) also reported some differences in the specificity of the peptidyl transferase of eukaryotic and prokaryotic systems. Therefore, a comparison between the activities of the different tRNAs modified at the terminal adenosine residue in ribosomal system derived from prokaryotic and eukaryotic cells is of great interest.

Materials and Methods

$tRNA^{Phe}$-C-C from yeast was prepared from baker's yeast bulk tRNA (Boehringer, Mannheim), which contained about 30% tRNA-C-C-A and 70% tRNA-C-C. The mixture of $tRNA^{Phe}$-C-C-A and $tRNA^{Phe}$-C-C obtained by chromatography on BD-cellulose (benzoylated DEAE-cellulose, Boehringer, Mannheim) was separated by chromatography on a Sephadex A-25 column according to Schneider et al. (1972). In the case of $tRNA^{Phe}$-C-C-A preparation, the 3′ end of the $tRNA^{Phe}$ fraction obtained by BD-cellulose chromatography was enzymatically regenerated, using tRNA nucleotidyl transferase (EC 2.7.7.25) from baker's yeast and then further purified by chromatography on Sephadex A-25. $tRNA^{Phe}$-C-C-A accepted 1470 pmol of phenylalanine/A_{260} unit of tRNA. $tRNA^{Phe}$-C-C accepted 1480 pmol of phenylalanine when tRNA nucleotidyl transferase was present in the aminoacylation assay, whereas in the absence of this enzyme aminoacylation did not take place. Aminoacylation assay as described by Sprinzl et al. (1973a) was used.

$tRNA^{Phe}$-C-C-3′dA was prepared as described previously (Sprinzl et al., 1973b) and was free of $tRNA^{Phe}$-C-C-A as could be shown by 3′-end group analysis (Sprinzl et al., 1972). Its phenylalanine acceptance was 1485 pmol/A_{260} unit of tRNA. $tRNA^{Phe}$-C-C-$A_{oxi-red}$ was obtained as described (Cramer et al., 1968; Chen and Ofengand, 1970) and accepted 1420 pmol of phenylalanine/A_{260} unit of tRNA. Prior to preparative aminoacylation, $tRNA^{Phe}$-C-C-$A_{oxi-red}$ and $tRNA^{Phe}$-C-C-3′dA were treated with sodium periodate under conditions which would quantitatively oxidize native $tRNA^{Phe}$-C-C-A (Cramer et al., 1968; Ofengand and Chen, 1972); traces of native $tRNA^{Phe}$-C-C-A would thereby be eliminated. $tRNA^{Phe}$-C-C-3′NH_2A from yeast was prepared in an analogous way to that described by Fraser and Rich (1973) for the *Escherichia coli* system, by incorporation of 3′-deoxy-3′-aminoadenosine 5′-phosphate into $tRNA^{Phe}$-C-C, using tRNA nucleotidyl transferase from yeast. Reaction conditions were those described previously (Sprinzl et al., 1973b) but the incubation was performed for 16 h at 30 °C. $tRNA^{Phe}$-C-C-3′NH_2A accepted 1520 pmol of phenylalanine/A_{260} unit of tRNA and did not contain any native $tRNA^{Phe}$-C-C-A. This was shown by 3′-end nucleoside analysis (Sprinzl et al., 1972) and by the fact that after aminoacylation the phenylalanine was bound by an amide bond, stable at alkaline conditions (Sprinzl et al., 1975).

$tRNA^{Phe}$ species were aminoacylated according to Sprinzl and Cramer (1973) with purified phenylalanyl-tRNA synthetase from yeast (EC 6.1.1.20; von der Haar, 1973) up to 1300–1500 pmol of phenylalanine/A_{260} unit of $tRNA^{Phe}$. The phenylalanyl-tRNA synthetase from yeast (a gift of Dr. F. von der Haar) was free of nuclease and tRNA nucleotidyl transferase activities as shown by its inability to aminoacylate $tRNA^{phe}$-C-C and $tRNA^{Phe}$-C-C-2′dA, respectively (Sprinzl et al., 1973b). Formation of Phe-$tRNA^{Phe}$-C-C-A during the enzymatic aminoacylation of modified $tRNA^{Phe}$ species could therefore be excluded.

Ac-Phe-$tRNA^{Phe}$ species and peptidyl-$tRNA^{Phe}$ species were prepared from the corresponding Phe-$tRNA^{Phe}$ species by the method of Lapidot and Rappoport (1974) except that the products were precipitated with alcohol instead of dichloroacetic acid.

Crude ribosomes from rabbit reticulocytes (Rahamimoff and Arnstein, 1969) were purified essentially according to Martin and Wool (1969) by incubating them consecutively in an amino acid incorporation mixture containing puromycin and in a medium containing 0.88 M KCl. These ribosomes are practically free of tRNA and have no endogenous mRNA activity. The binding of radioactive Phe-$tRNA^{Phe}$ species to ribosomes was determined by the Millipore filter technique of Nirenberg and Leder (1964). Standard buffer for binding studies contained 60 mM Tris-HCl (pH 7.4), 70 mM KCl, 1 mM dithiothreitol, and a $MgCl_2$ concentration as specified. Binding of nonaminoacylated tRNA to ribosomes was determined by a procedure described by Koka and Nakamoto (1974) as follows. The nonaminoacylated tRNA was allowed to bind to the ribosomes, then the incubation mixture was filtered through a Millipore filter (HA 0.45 μm) and the filter was washed extensively with washing buffer containing the same magnesium ion concentration as the incubation mixture. The ribosome-bound tRNA was then eluted quantitatively by washing the filter three times with 0.7 ml of washing buffer not containing magnesium ions. The tRNA content in the combined eluates was determined by measuring the amino acid acceptance activity of the appropriate sample.

Donor activities of the corresponding modified Ac-Phe-$tRNA^{Phe}$ species were measured by the puromycin reaction, as described by Leder and Bursztyn (1966). Acceptor activity was determined by measuring the formation of Ac-$(Phe)_2$-$tRNA^{Phe}$ from the various Phe-$tRNA^{Phe}$ species and prebound Ac-Phe-$tRNA^{Phe}$-C-C-A.

Isolation of ribosome-bound radioactive material for anal-

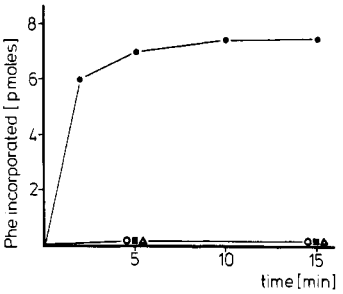

FIGURE 2: Poly(U)-directed poly(Phe) synthesis. Incubation mixture (final volume 0.1 ml) contained standard buffer with 6 mM $MgCl_2$, 40 μg of poly(U), 0.5 mM GTP, 0.5 A_{260} unit of ribosomes, 100 μg of protein (40–70% ammonium sulfate precipitate of the S-100 fraction), and 30 pmol of the different [^{14}C]Phe-tRNA^{Phe} species. Incubation was performed at 37 °C for the period of time indicated. (●—●) [^{14}C]Phe-tRNA^{Phe}-C-C-A; (○—○) [^{14}C]Phe-tRNA^{Phe}-C-C-3′dA; (■—■) [^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$; (△—△) [^{14}C]Phe-tRNA^{Phe}-C-C-3′NH$_2$A.

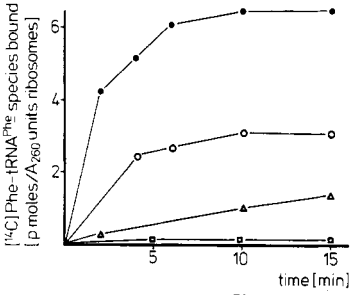

FIGURE 3: Enzymatic binding of Phe-tRNA^{Phe} species to ribosomes. Each incubation mixture (50 μl) contained standard buffer with 6 mM $MgCl_2$, 40 μg of poly(U), 1.0 A_{260} unit of ribosomes, 25 pmol of the [^{14}C]Phe-tRNA^{Phe} species, 0.5 mM GTP, and 15 μg (protein) of EF-I. Incubation was carried out at 37 °C. The results shown are those obtained experimentally after subtracting the binding values obtained in the absence of GTP and EF-I. (●—●) [^{14}C]Phe-tRNA^{Phe}-C-C-A; (○—○) [^{14}C]Phe-tRNA^{Phe}-C-C-3′dA; (□—□) [^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$; (△—△) [^{14}C]Phe-tRNA^{Phe}-C-C-3′NH$_2$A.

ysis of the dipeptide formation was performed as described by Siler and Moldave (1969). Alkaline hydrolysis of the formed peptidyl-tRNA derivatives was carried out with 0.5 N NaOH at 37 °C for 1 h. Radioactive products were separated by paper chromatography, using the solvent system specified.

Acceptor activity of Phe-tRNA^{Phe}-C-C-3′NH$_2$A was determined as described by Fraser and Rich (1973). To 2.0 A_{260} units of ribosomes carrying 26 pmol of prebound Ac-[^{14}C]-Phe-tRNA^{Phe}-C-C-A, 48 pmol of [^{3}H]Phe-tRNA^{Phe}-C-C-3′NH$_2$A was added under enzymatic (20 μg EF-I, 6 mM $MgCl_2$, and 0.5 mM GTP) or nonenzymatic (20 mM $MgCl_2$) binding conditions. After 10 min at 37 °C Tris base was added to a final concentration of 1 M (pH 9.0) and the mixture was incubated for a further 2 h at 37 °C. The radioactivity of the cold 5% trichloroacetic acid insoluble material was counted. Poly(U) directed polyphenylalanine synthesis was determined according to Bollum (1965) by measuring the incorporation of [^{14}C]phenylalanine into hot 10% trichloroacetic acid insoluble material, using Whatman 3MM filter paper.

EF-I and EF-II from rat liver were a generous gift of Dr. K. Moldave, University of California, Irvine, California. tRNA-nucleotidyl transferase from yeast was a gift of Dr. H. Sternbach, Max-Planck-Institut für experimentelle Medizin, Göttingen, Germany.

[^{14}C]L-Phenylalanine (405 mCi/mmol) and [^{3}H]-L-phenylalanine (25 Ci/mmol) were obtained from Amersham-Buchler, Radiochemikalien, Braunschweig, Germany.

Results

The poly(U) dependent incorporation of [^{14}C]phenylalanine into 5% trichloroacetic acid insoluble peptides catalyzed by a reticulocyte ribosomal system was investigated with the following substrates: [^{14}C]Phe-tRNA^{Phe}-C-C-A, [^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$, [^{14}C]Phe-tRNA^{Phe}-C-C-3′dA, and [^{14}C]Phe-tRNA^{Phe}-C-C-3′NH$_2$A (Figure 1).

As can clearly be seen from Figure 2, only the unmodified [^{14}C]Phe-tRNA^{Phe} could serve as a substrate in this assay, whereas the [^{14}C]Phe from the modified Phe-tRNA^{Phe} species was not incorporated into oligopeptides. Oligo-Phe-tRNA^{Phe} formation from Phe-tRNA^{Phe} is the result of a sequence of several well-defined steps. The lack of formation of oligo-Phe-tRNA^{Phe} from Phe-tRNA^{Phe} species with a modified 3′-ribose residue may be due to the inability of these substrates to take part in one or more of those steps. The activities of the modified tRNAs in the particular steps were therefore studied in detail.

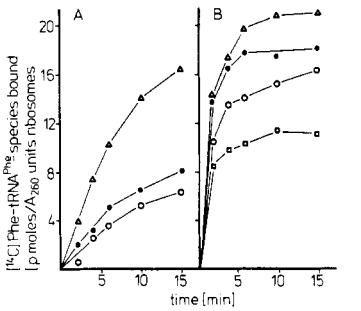

FIGURE 4: Kinetics of nonenzymatic binding of Phe-tRNA^{Phe} species to ribosomes. Reaction mixture (final volume 0.1 ml) contained standard buffer with either 6 mM $MgCl_2$ (A) or 20 mM $MgCl_2$ (B), 40 μg of poly(U), 0.5 A_{260} unit of ribosomes, and 25 pmol of the different [^{14}C]Phe-tRNA^{Phe} species. (●—●) [^{14}C]Phe-tRNA^{Phe}-C-C-A; (○—○) [^{14}C]Phe-tRNA^{Phe}-C-C-3′dA; (□—□) [^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$; (△—△) [^{14}C]Phe-tRNA^{Phe}-C-C-3′NH$_2$A.

The rate of the enzymatic binding of the different substrates to 80S ribosomes is shown in Figure 3. The values presented are corrected for the binding observed in the absence of EF-I and represent a real stimulation of the binding by the binding factor. The EF-I used in these experiments was a highly purified preparation from rat liver. It is clear from the results in Figure 3 that the rat liver EF-I binds unmodified Phe-tRNA^{Phe} from yeast very effectively to reticulocyte ribosomes. Phe-tRNA^{Phe}-C-C-3′dA binding is stimulated to a lower extent but Phe-tRNA^{Phe}-C-C-3′NH$_2$A binding is even less catalyzed. Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$ is not a substrate in the EF-I dependent binding to the 80S ribosomes.

The kinetics of the nonenzymatic binding of Phe-tRNA^{Phe} species at the same magnesium ion concentration as used in the enzymatic binding (6 mM) is shown in Figure 4A. It is surprising that the binding of Phe-tRNA^{Phe}-C-C-3′NH$_2$A is much faster than that of the unmodified Phe-tRNA^{Phe}. Phe-tRNA^{Phe}-C-C-3′dA shows nearly the same rate and extent of nonenzymatic binding as the native Phe-tRNA^{Phe} at this magnesium concentration. At high magnesium ion concentration (20 mM, Figure 4B) the extent of binding of these substrates seems not to differ significantly. Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$ which failed in the enzymatic binding can be bound to ribosomes at this magnesium concentration.

The difference in nonenzymatic binding behavior of Phe-tRNA^{Phe} and Phe-tRNA^{Phe}-C-C-3′NH$_2$A is strongly dependent upon the magnesium ion concentration (Table I).

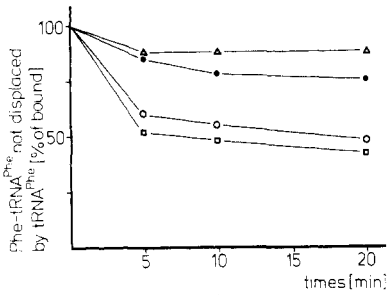

FIGURE 5: Displacement of nonenzymatically bound Phe-tRNA^{Phe} species by tRNA^{Phe}-C-C-A. Seventy-five picomoles of [^{14}C]Phe-tRNA^{Phe} species were incubated with 4.0 A_{260} units of ribosomes and 200 μg of poly(U) in the standard buffer with 20 mM $MgCl_2$. After 15 min incubation at 37 °C, 0.3 A_{260} unit of tRNA^{Phe}-C-C-A was added. Samples were withdrawn at the times indicated and the remaining amount of Phe-tRNA^{Phe} species bound to ribosomes was determined. In control experiments, where no tRNA^{Phe}-C-C-A was added, the amount of the different Phe-tRNA^{Phe} species bound in the first 15 min remained in all cases constant during the additional incubation (not shown). (●—●) Phe-tRNA^{Phe}C-C-A; (○—○) Phe-tRNA^{Phe}-C-C-3′dA; (□—□) Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$; (△—△) Phe-tRNA^{Phe}-C-C-3′NH_2A.

TABLE I: Magnesium Concentration Dependence of the Nonenzymatic Binding of [^{14}C]Phe-tRNA^{Phe}-C-C-A and [^{14}C]-Phe-tRNA^{Phe}-C-C-3′NH_2A to the Ribosomes.[a]

tRNA^{Phe} Species	Mg^{2+} Concn (mM)				
	3	6	10	15	20
[^{14}C]Phe-tRNA^{Phe}-C-C-A	0.2	2.9	9.3	12.3	12.3
[^{14}C]Phe-tRNA^{Phe}-C-C-3′NH_2A	1.3	7.6	11.9	16.2	16.2

[a] Reaction mixture (final volume 0.1 ml) contains: standard buffer with the indicated amount of $MgCl_2$, 40 μg of poly(U), 1.0 A_{260} unit of ribosomes, and 25 pmol of [^{14}C]Phe-tRNA^{Phe}-C-C-A or [^{14}C]Phe-tRNA^{Phe}-C-C-3′NH_2A. The binding was determined after 10 min of incubation at 37 °C.

Whereas at 3 mM magnesium the difference in the extent of the binding is at least fivefold in favor of the modified tRNA, at higher magnesium concentration the difference almost disappears.

Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$ and Phe-tRNA^{Phe}-C-C-3′dA, bound nonenzymatically to the ribosomes, can be displaced by nonaminoacylated, native tRNA^{Phe}. The complexes formed with Phe-tRNA^{Phe} and Phe-tRNA^{Phe}-C-C-3′NH_2A under similar conditions are much more stable (Figure 5).

For investigation of the donor activity of the modified tRNAs the corresponding Ac[^{14}C]Phe-tRNA^{Phe} species were prepared. These were bound nonenzymatically to reticulocyte ribosomes and the puromycin reaction was measured (Table II). Although 40% of the bound Ac-Phe-tRNA^{Phe}-C-C-A reacted with puromycin none of the modified Ac-[^{14}C]Phe-tRNA^{Phe}s were active as donors of the peptidyl residue. Even after the addition of the translocation factor EF-II no reaction could be detected between puromycin and the modified Ac-Phe-tRNA^{Phe} species, while under these conditions 80% of the bound Ac-Phe-tRNA^{Phe}-C-C-A reacted with puromycin.

In order to prove that the failure of the modified Ac-Phe-tRNAs to react with puromycin is not due to the inability of these substrates to bind to the ribosomal donor site an experiment summarized in Table III was carried out. As can be seen from these results the enzymatic binding of Phe-tRNA^{Phe}-C-C-A is inhibited by preincubating the ribosomes with Ac-Phe-tRNA^{Phe}-C-C-A. The reason for this effect is the fact, that under these conditions, besides the binding to the P-site about 40% of the total bound Ac-Phe-tRNA^{Phe}-C-C-A is at the ribosomal A-site.

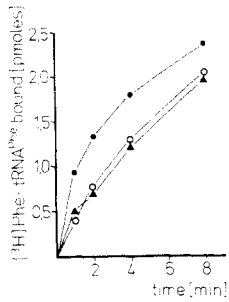

FIGURE 6: Kinetics of enzymatic binding of Phe-tRNA^{Phe} to ribosomes with peptidyl-tRNA on their P site. Reaction mixture contained (in 0.165 ml) standard buffer with 20 mM $MgCl_2$, 400 μg poly(U), 10 A_{260} units of ribosomes and, when indicated, 90 pmol of (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-A or (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-3′dA. After incubation at 37 °C for 8 min, 15 μg of EF-II and GTP to a concentration of 0.5 mM were added. After additional incubation for 10 min at 37 °C the reaction mixtures were cooled to 0 °C and treated for 10 min at 37 °C with 10 mM *N*-ethylmaleimide to destroy EF-II. Mercaptoethanol was then added to a final concentration of 20 mM and allowed to react for 5 min at 0 °C to destroy the excess *N*-ethylmaleimide. Reaction mixtures were further incubated at 16 °C and 9 μg of EF-I and 50 pmol of [^{3}H]Phe-tRNA^{Phe}-C-C-A (specific activity 1 Ci/mmol) were added (the final volume was 0.5 ml; $MgCl_2$ concentration was adjusted to 6 mM). Samples (0.1 ml) were withdrawn at the times indicated and the binding of [^{3}H]Phe-tRNA^{Phe}-C-C-A was determined. (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-A and (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-3′dA were bound to the same extent (4.5 pmol/A_{260} unit of ribosomes). (●—●) (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-A; (△—△) (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-3′dA; (○—○) ribosomes without peptidyl-tRNA.

As can be seen from Table III the preincubation of the ribosomes with the modified Ac-Phe-tRNAs also inhibits the enzymatic binding of Phe-tRNA^{Phe}-C-C-A. These results are in good agreement with the calculated, expected values which are obtained on the assumption that the distribution of the bound modified Ac-Phe-tRNA^{Phe} species between the two ribosomal binding sites, is similar to that of Ac-Phe-tRNA^{Phe}-C-C-A. Should the modified Ac-Phe-tRNA^{Phe} species not bind at all to the donor site, but only to the acceptor site, the enzymatic binding of Phe-tRNA^{Phe}-C-C-A would be much lower than that shown in Table III.

It was previously shown that donor site bound peptidyl-tRNA facilitates the binding of aminoacyl-tRNA to the acceptor site. Nonaminoacylated tRNA has no such effect (Baksht and de Groot, 1974). We therefore compared the effect of P-site bound unmodified peptidyl-tRNA^{Phe} on the kinetics of the binding of the Phe-tRNA^{Phe} with the effects of P-site bound modified peptidyl-tRNA^{Phe} species on this binding reaction. Figure 6 shows the kinetics of the enzymatic binding of Phe-tRNA^{Phe}-C-C-A to three different populations of ribosomes: ribosomes preincubated with (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-A and poly(U); ribosomes preincubated with (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-3′dA and poly(U); and the ribosomes preincubated with poly(U) alone. The enzymatic binding of Phe-tRNA^{Phe}-C-C-A to the first mentioned ribosomes is faster than the binding to ribosomes preincubated with poly(U) alone. There is, however, no difference between the kinetics of Phe-tRNA^{Phe} binding to ribosomes preincubated with (Gly)$_3$-[^{14}C]Phe-tRNA^{Phe}-C-C-3′dA and the kinetics of Phe-tRNA^{Phe} binding to ribosomes preincubated without a peptidyl-tRNA.

TABLE II: Donor Activity of Ac-Phe-tRNA^{Phe} Species.[a]

Substrates	Amount of Substrate Bound (pmol)	Ac-Phe-puromycin Formed			
		Without EF II		With EF II	
		pmol	% of Bound	pmol	% of Bound
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-A	7.0	2.7	40	5.7	80
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-3′NH$_2$A	6.9	0.0		0.0	
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-3′dA	3.3	0.0		0.0	
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$	2.4	0.0		0.0	

[a] Reaction mixture (0.4 ml) contained: standard buffer with 20 mM $MgCl_2$, 160 μg of poly(U), 4.0 A_{260} units of ribosomes, and 60 pmol of the different Ac-[^{14}C]Phe-tRNA species. After 15 min at 37 °C, the binding was determined in aliquots of 100 μl. The extent of the puromycin reaction with each of the substrates was determined in two 100-μl aliquots after puromycin (final concentration 0.5 mM) was added and followed by an additional incubation period of 20 min. To one of the aliquots 30 μg of EF-II and GTP (final concentration 0.5 mM) was also added.

TABLE III: Enzymatic Binding of [^{3}H]Phe-tRNA-C-C-A to Ribosomes with Prebound Ac-[^{14}C]Phe-tRNA^{Phe} Species.[a]

Species of Ac-[^{14}C]Phe-tRNA^{Phe} Prebound in First Incubation	[^{3}H]Phe-tRNA^{Phe}-C-C-A Added	Amount of Ac-[^{14}C]Phe-tRNA^{Phe} Bound (pmol)	Amount of [^{3}H]Phe-tRNA^{Phe}-C-C-A Bound (pmol)
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-A	No	6.3	
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-3′NH$_2$A	No	7.1	
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-3′dA	No	3.8	
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$	No	2.9	
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-A	Yes	5.3	2.6
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-3′NH$_2$A	Yes	7.4	2.0
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-3′dA	Yes	4.3	3.6
Ac-[^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$	Yes	2.2	4.8
None	Yes	-	6.0

[a] Incubation mixtures (0.1 ml) containing standard buffer with 20 mM $MgCl_2$, 60 μg of poly(U), 1.0 A_{260} unit of ribosomes, and 45 pmol of the different Ac-[^{14}C]Phe-tRNA^{Phe} species were incubated at 37 °C and to some of them 30 pmol of [^{3}H]Phe-tRNA^{Phe}-C-C-A, 35 μg of EF-I, and GTP (final concentration 0.5 mM) were added after 10 min. Ten minutes later the samples were assayed for Ac-[^{14}C]Phe-tRNA^{Phe} species and [^{3}H]Phe-tRNA^{Phe} bound to the ribosomes.

The acceptor activity of the modified Phe-tRNA^{Phe} species was measured in different ways. In the case of Phe-tRNA^{Phe}-C-C-3′dA, the Ac-[^{3}H]Phe-tRNA^{Phe}-C-C-A was first bound to the ribosomes and in the second step [^{14}C]Phe-tRNA^{Phe}-C-C-3′dA was added under enzymatic binding conditions. As can be concluded from the appearance of Ac-Phe-Phe in the chromatogram of the alkaline hydrolysate of the ribosomal complex, dipeptide formation took place (Figure 7A). In the case of Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$, where the ribosomes were first incubated with Ac-[^{3}H]Phe-tRNA^{Phe} and then [^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$ was bound in the absence of EF-I at 20 mM magnesium concentration, no peptide bond formation could be detected, although a considerable binding of [^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$ took place. Under the same conditions unmodified Phe-tRNA^{Phe} gave rise to the formation of Ac-Phe-Phe-tRNA (Figure 7B).

In the case of Phe-tRNA^{Phe}-C-C-3′NH$_2$A the acceptor activity has to be determined by a different method since the amino acid is bound to this tRNA by a stable amide bond. This alkaline stability (Fraser and Rich, 1973) was used as a basis for providing the evidence for the acceptor activity of Phe-tRNA^{Phe}-C-C-3′NH$_2$A (Experimental details are given under Materials and Methods). When [^{3}H]Phe-tRNA^{Phe}-C-C-3′NH$_2$A was bound to ribosomes carrying prebound Ac-[^{14}C]Phe-tRNA (26 pmol) under enzymatic binding conditions, 2.50 pmol of Ac-[^{14}C]Phe was found linked to the tRNA^{Phe}-C-C-3′NH$_2$A through an alkali stable bond. If the binding was done under nonenzymatic binding conditions, 1.40 pmol of Ac[^{14}C]Phe was transferred to the modified tRNA in the A-site. The values are those obtained experimentally after the appropriate blank values were subtracted. These results clearly indicate that Phe-tRNA^{Phe}-C-C-3′NH$_2$A is able to accept the peptidyl residue and that the presence of EF-I facilitates the proper binding of [^{3}H]Phe-tRNA^{Phe}-C-C-3′-NH$_2$A to the A-site.

It is known that the nonaminoacylated tRNA binds with a high affinity to eukaryotic ribosomes. As shown in Table IV the nonaminoacylated tRNA^{Phe} species which possess a modification on the 3′ position of the terminal ribose, tRNA^{Phe}-C-C-3′NH$_2$A and tRNA^{Phe}-C-C-3′dA, have in the absence of poly(U) a similar binding affinity as the native tRNA^{Phe}-C-C-A. The binding of tRNA^{Phe}-C-C-A$_{oxi-red}$ is considerably lower. The binding of aminoacyl-tRNA is under these conditions much lower. By addition of poly(U) the binding of unmodified tRNA^{Phe}-C-C-A is stimulated at least twice as much as the binding of tRNA^{Phe}-C-C-3′NH$_2$A or tRNA^{Phe}-C-C-3′dA. The binding of tRNA^{Phe}-C-C-A$_{oxi-red}$ in the presence of poly(U) is about the same as that of the other two modified tRNA^{Phe} species investigated.

Because of the high binding affinity of the nonaminoacylated tRNAs to the ribosomes, it seemed interesting to measure the binding of tRNA^{Phe}-C-C and tRNA^{Phe}-C-C-2′dA, which cannot be aminoacylated (Sprinzl and Cramer, 1973) to the ribosomes. A suitable experimental assay for this purpose is described in Figure 8. When Phe-tRNA^{Phe}-C-C-A is added to the reticulocyte ribosomes a considerable part (up to 50%) of the bound Phe-tRNA^{Phe} is converted to Phe-Phe-tRNA^{Phe}. Formation of the dipeptide is very sensitive to inhibition by

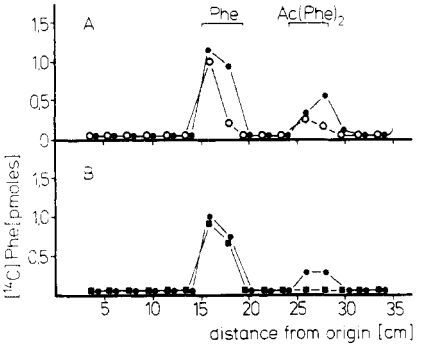

FIGURE 7: Acceptor activity of Phe-tRNA^{Phe}-C-C-3′dA and Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$. Reaction was carried out in two steps. In the first step Ac-[^{3}H]Phe-tRNA^{Phe}-C-C-A was bound to ribosomes in the standard buffer containing 20 mM $MgCl_2$. To 2 A_{260} units of ribosomes carrying 13 pmol of Ac-[^{3}H]Phe-tRNA^{Phe}-C-C-A, 75 pmol of the different [^{14}C]Phe-tRNA^{Phe} species was added in the second step. This was performed: (A) with 17 μg of EF-I, 1 mM GTP, and 6 mM $MgCl_2$; (B) with 20 mM $MgCl_2$. The second incubation was carried out for 15 min at 37 °C. Ribosomes were isolated by filtration through nitrocellulose filters. Ribosomal bound ^{14}C-labeled products were eluted with sodium dodecyl sulfate and analyzed as described by Siler and Moldave (1969). Chromatography solvent system was 1-butanol saturated with 2 M NH_4OH. (●—●) [^{14}C]Phe-tRNA^{Phe}-C-C-A; (○—○) [^{14}C]Phe-tRNA^{Phe}-C-C-3′dA; (■—■) [^{14}C]Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$.

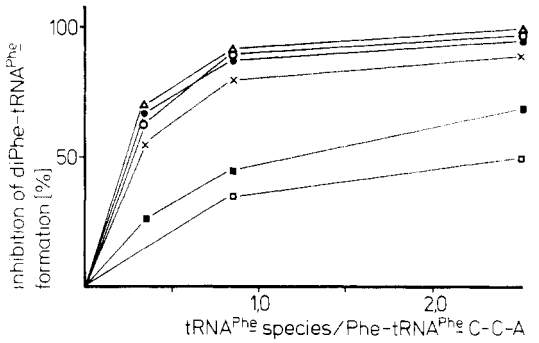

FIGURE 8: Inhibition of diPhe-tRNA^{Phe} formation by nonaminoacylated tRNAs. Reaction mixture containing standard buffer with 20 mM $MgCl_2$, 100 μg of poly(U), 2 A_{260} units of ribosomes, 50 pmol of [^{14}C]Phe-tRNA^{Phe}-C-C-A, and the indicated amount of different tRNA^{Phe} species was incubated for 15 min at 37 °C. Reaction mixture was filtered through nitrocellulose filters and the reactive ribosomal bound material was analyzed as described in the legend to Figure 7. Results are expressed as percent inhibition of diPhe-tRNA^{Phe} formation by nonaminoacylated tRNAs. (●—●) tRNA^{Phe}-C-C-A; (○-○) tRNA^{Phe}-C-C-3′dA; (△—△) tRNA^{Phe}-C-C-3′NH$_2$A; (X—X) tRNA^{Phe}-C-C-2′dA; (■--■) tRNA^{Phe}-C-C-A$_{oxi-red}$; (□—□) tRNA^{Phe}-C-C.

TABLE IV: Binding of Nonaminoacylated tRNA^{Phe} Species to Ribosomes.[a]

tRNA^{Phe}	Binding (pmol/A_{260} unit of Ribosomes)	
	Without Poly(U)	With Poly(U)
tRNA^{Phe}-C-C-A	2.7	12.7
tRNA^{Phe}-C-C-3′NH$_2$A	3.3	5.7
tRNA^{Phe}-C-C-3′dA	3.1	6.2
tRNA^{Phe}-C-C-A$_{oxi-red}$	0.9	4.0
[^{14}C]Phe-tRNA^{Phe}-C-C-A	0.1	1.9

[a] Reaction mixture (final volume 0.1 ml) contained standard buffer with 8 mM $MgCl_2$, 60 μg of poly(U), 1.0 A_{260} unit of ribosomes, 150 pmol of tRNA^{Phe} species, or [^{14}C]Phe-tRNA^{Phe}-C-C-A. Incubation was carried out for 15 min at 37 °C. Binding of nonaminoacylated tRNA was determined as described under Methods.

nonaminoacylated tRNA, which is probably due to competition between nonaminoacylated tRNA and Phe-tRNA^{Phe} for P-site occupancy (Rahamimoff et al., 1972). The ability of the modified tRNA^{Phe} species to inhibit the Phe-Phe-tRNA^{Phe} formation was therefore determined (Figure 8). tRNA^{Phe}-C-C-3′NH$_2$A and tRNA^{Phe}-C-C-3′dA are as powerful inhibitors of the dipeptidyl-tRNA formation as unmodified tRNA^{Phe}, whereas the inhibitory effect of tRNA^{Phe}-C-C-A$_{oxi-red}$ and of tRNA^{Phe}-C-C is lower. tRNA^{Phe}-C-C-2′dA inhibits the Phe-Phe-tRNA formation nearly as effectively as the unmodified tRNA^{Phe}.

Discussion

In the cell-free polyphenylalanine synthesizing system from rabbit reticulocytes, not one of the modified Phe-tRNAs (Figure 1) was able to incorporate its amino acid into a polypeptide. This is similar to the results obtained with the *E. coli* system where the Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$ (Ofengand and Chen, 1972; Chinali et al., 1974), Phe-tRNA^{Phe}-C-C-3′dA (Chinali et al., 1974), and Phe-tRNA^{Phe}-C-C-3′NH$_2$A (Fraser and Rich, 1973) are also inactive in the poly(U)dependent poly(Phe) synthesis. This failure can be explained by the observation that in both systems, the prokaryotic (*E. coli*) as well as the eukaryotic one (reticulocytes), all three Ac-Phe-tRNA^{Phe} species derived from the modified tRNAs have no donor activity (Table II). However, from these findings one cannot draw a final conclusion about the 2′-, 3′-specificity of the donor site. The possibility exists that either the 2′-aminoacyl-tRNA is the normal substrate for the peptidyl transferase at the donor site but the vicinal 3′-hydroxyl group is necessary for the enzyme action, or that the 3′-aminoacyl-tRNA is the normal substrate at the donor site, but the peptidyl transferase cannot cleave the amide bond by which the peptidyl residue is linked to the terminal ribose of the Phe-tRNA^{Phe}-C-C-3′NH$_2$A. In this connection it may be of interest that the peptidyl-tRNA hydrolase from *E. coli* cleaves the peptidyl residue from peptidyl-tRNA^{Phe}-C-C-A and peptidyl-tRNA^{Phe}-C-C-3′NH$_2$A at nearly the same rate (Shiloach et al., 1975).

Experiments in Table III suggest that the absence of donor activity of the modified peptidyl-tRNAs is not related to their inability to bind to the ribosomal P-site. In these experiments the enzymatic binding of Phe-tRNA^{Phe}-C-C-A to the ribosomes preincubated with modified Ac-Phe-tRNA^{Phe} species was investigated. The results can best be explained by the assumption that under non-enzymatic conditions the distribution of our modified Ac-Phe-tRNA^{Phe} species between the donor and acceptor sites is similar to that of distribution of the unmodified Ac-Phe-tRNA^{Phe}-C-C-A, which binds equally to both sites. There are, however, differences between the unmodified and modified species in the efficiency of the interaction with the ribosomes. The Phe-tRNA^{Phe}-C-C-3′dA and Phe-tRNA^{Phe}-C-C-A$_{oxi-red}$, which were bound to ribosomes under nonenzymatic conditons are more easily displaced by a nonaminoacylated tRNA^{Phe} than the native Phe-tRNA^{Phe}-C-C-A (Figure 5). Since the tRNA^{Phe} can replace only a part of the bound modified Phe-tRNA^{Phe} species, it is most likely that this exchange takes place at the donor site only. Phe-tRNA^{Phe}-C-C-3′NH$_2$A, which was also investigated in

this experiment, is exchanged to an even lower extent than the Phe-tRNA^{Phe}-C-C-A. This indicates that the aminoacyl-tRNA on which the aminoacyl residue is on the 3′-position of the terminal ribose interacts effectively with the donor site of the ribosomes. In contrast, the Phe-tRNA^{Phe}-C-C-3′dA being aminoacylated on the 2′-position has a lower affinity for this site. Similar conclusions can be drawn from the experiments shown in Figure 6. Peptidyl-tRNA-C-C-A bound to the donor site stimulates the EF-I-dependent binding of aminoacyl-tRNA to the acceptor site. This is probably due to an induction of a conformational change in which the ribosomal components participating in the binding of the EF-I·aminoacyl-tRNA·GTP complex are involved (Baksht and de Groot, 1974). Peptidyl-tRNA^{Phe}-C-C-3′dA does not show such stimulatory effect. Probably its 3′ terminus, carrying the peptidyl residue on the wrong position, cannot be bound to the donor site in a way, which is necessary to facilitate the binding of aminoacyl-tRNA to the acceptor site. It is possible that the ability of the donor site bound peptidyl-tRNA to induce a conformational change in the ribosome is a property of the donor substrate.

Summarizing the results concerning the interactions of aminoacyl- or peptidyl-tRNA with the donor site of reticulocyte ribosomes, we can conclude that the peptidyl residue of the tRNA-C-C-A bound to this site is linked most probably to the 3′ position of the terminal adenosine.

Other findings related to the tRNA–ribosome interaction at the donor site are shown in Table IV. Nonaminoacylated tRNA^{Phe}-C-C-A binds with high affinity to the reticulocyte ribosomes even in the absence of the appropriate messenger RNA. The modified tRNA^{Phe}-C-C-3′dA and tRNA^{Phe}-C-C-3′NH_2A bind with the same efficiency. tRNA^{Phe}-C-C-$A_{oxi-red}$, on the other hand, shows a considerable lower binding under these conditions. It is, however, rather striking that poly(U) stimulates the binding of the modified tRNA^{Phe} species to a much lower extent than the binding of the tRNA^{Phe}-C-C-A. These results may be explained by assuming a cooperative effect in tRNA–mRNA–ribosome interaction. Optimal stimulation of the tRNA binding by a correct codon–anticodon interaction is probably dependent on the accurate fit between the -C-C-A terminus of the tRNA and the ribosome. The 3′ ends of the modified tRNA^{Phe} species seem to interact in a different way with the peptidyl transferase than the unmodified tRNA. This difference becomes, however, apparent only when the fidelity of the 3′-terminus recognition is increased by the correct codon–anticodon interaction. The stimulation of the modified tRNA^{Phe} species binding by poly(U) is therefore smaller than that of unmodified tRNA^{Phe}.

The importance of the complete 3′ terminus of the nonaminoacylated tRNA^{Phe} for binding to the donor site of the reticulocyte ribosomes can also be demonstrated by experiments in Figure 8. Here the absence of the 3′-terminal adenosine of the tRNA^{Phe} or a severe structural change on this nucleoside due to cleavage of the C(2′)–C(3′) carbon bond (Maelicke et al., 1974) weakens the binding of tRNA^{Phe}-C-C or tRNA^{Phe}-C-C-$A_{oxi-red}$, respectively, to the ribosomes, leading to decrease in the inhibitory activity of these tRNAs on di-Phe-tRNA^{Phe} formation as compared with native tRNA^{Phe}-C-C-A. As this inhibition of Phe-Phe-tRNA^{Phe} formation is probably caused by a direct binding of the -C-C-A terminal sequence of the inhibiting tRNA to the donor site of the peptidyl transferase, the experiments in Figure 8 show that the tRNA^{Phe}-C-C-3′dA and tRNA^{Phe}-C-C-3′NH_2A bind with a similar affinity to the donor site as the native tRNA^{Phe}-C-C-A.

The behavior of the modified Phe-tRNA^{Phe} species in the EF-I dependent binding to 80S ribosomes is very similar to the case of EF-Tu promoted binding of these tRNAs to *E. coli* ribosomes (Chen and Ofengand, 1970; Ofengand and Chen, 1972; Chinali et al., 1974; Sprinzl et al., 1975). Phe-tRNA^{Phe}-C-C-3′dA binding is stimulated by EF-I whereas the Phe-tRNA^{Phe}-C-C-$A_{oxi-red}$ is not active in this reaction. Only a little stimulation of the binding by EF-I was observed in the case of Phe-tRNA^{Phe}-C-C-3′NH_2A, but in spite of the high blank values due to nonenzymatic binding of Phe-tRNA^{Phe}-C-C-3′NH_2A the results presented in Figure 3 are very reproducible. EF-I-dependent binding of Phe-tRNA^{Phe}-C-C-3′NH_2A to the A-site can be more clearly demonstrated by the twofold stimulation of the di-Phe-tRNA^{Phe}-C-C-3′NH_2A formation when the modified acceptor tRNA was bound in the presence of EF-I and at 6 mM magnesium concentration, as compared with the case when no EF-I was present and the magnesium concentration was 20 mM. If Phe-tRNA^{Phe}-C-C-3′NH_2A could not undergo enzymatic binding, an opposite result should be expected since the nonenzymatic binding of this tRNA is at 20 mM magnesium concentration twice as high as at 6 mM. Clearly the determination of the A-site bound aminoacyl-tRNA by its acceptor activity gives a more precise information about the amount of tRNA located in the A-site and about the stimulation of the A-site directed binding by EF-I.

Phe-tRNA^{Phe}-C-C-3′NH_2A shows no activity in the EF-Tu dependent binding to *E. coli* ribosomes (Sprinzl et al., 1975). It was suggested recently that, during the interaction of aminoacyl-tRNA with the elongation factor Tu, the amino acid is linked to the 2′-hydroxyl group of the terminal adenosine (Ringer and Chládek, 1975). Our results, however, disprove this hypothesis for the eukaryotic system. It is even possible that the lower efficiency of the 3′-aminoacylated Phe-tRNA^{Phe}-C-C-3′NH_2A to participate in the EF-I-dependent binding is caused by the unnatural amide bond by which the amino acid is linked to this tRNA and not by the position of the amino acid attached. Therefore, as in the case of the *E. coli* system we have to postulate that either the 2′-aminoacyl-tRNA is the right substrate for EF-I or this does not differentiate between 2′- or 3′-aminoacylated species.

The maximal binding achieved under enzymatic conditions is much lower for Phe-tRNA^{Phe}-C-C-3′dA than for Phe-tRNA^{Phe}-C-C-A. It is possible to explain this by the observation made by Chinali et al. (1974) who found that the ribosomal complex formed enzymatically with Phe-tRNA^{Phe}-C-C-3′dA is less stable than the corresponding complex with the unmodified Phe-tRNA^{Phe}-C-C-A. As suggested previously (Sprinzl et al., 1975) if the aminoacyl residue is not able to undergo a transacylation to the 3′ position of the terminal adenosine, the ribosomal complex formed with Phe-tRNA^{Phe}-C-C-3′dA dissociates. Thus transacylation is a necessary step in the enzymatic binding process.

The extent of the nonenzymatic binding of the Phe-tRNA^{Phe}-C-C-3′NH_2A to reticulocyte ribosomes is higher than that of the native substrate, Phe-tRNA^{Phe}-C-C-A. This difference which is more pronounced at low magnesium concentrations indicates a direct involvement of magnesium ions in the interaction between the -C-C-A terminal nucleotides and the ribosomal components. It is interesting to note that the binding of Phe-tRNA^{Phe}-C-C-3′NH_2A to *E. coli* ribosomes is only slightly higher than that of Phe-tRNA^{Phe}-C-C-A even at low magnesium concentration. This may indicate a difference in the mode of interaction between *E. coli* and reticulocyte ribosomes with aminoacyl-tRNA.

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