# Binding of Radioactive Oligonucleotides to Ribosomes*

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ABSTRACT: Aminoacyl-tRNA markedly stimulates the binding of [$^3$H]oligonucleotides of chain length 3–7 to ribosomes. Oligonucleotide binding to ribosomes is affected by putrescine, spermidine, and $Mg^{2+}$. Ribosomal-bound trinucleotides and aminoacyl-tRNA exchange with unbound trinucleotides and aminoacyl-tRNA; the rate of trinucleotide exchange is more rapid than that of aminoacyl-tRNA. Thus, ribosomal-bound aminoacyl-tRNA acts as a template for a set of synonym codons corresponding to the tRNA anticodon. Trinucleotide exchange therefore provides a means of elucidating the specificity of tRNA anticodons for mRNA codons with unfractionated tRNA preparations.

Many aspects of codon recognition and protein synthesis have been studied by determining the binding of radioactive aa-tRNA[1] to ribosomes; however, little information is available on the binding of radioactive trinucleotides to ribosomes other than those required for the termination of protein synthesis (Brown *et al.*, 1968; Goodman *et al.*, 1968; Caskey *et al.*, 1969; Capecchi and Klein, 1969; Hatfield, 1966).

In the present study the characteristics of [$^3$H]trinucleotide binding to ribosomes in response to aa-tRNA and factors that influence both the association and dissociation of radioactive oligonucleotides and aa-tRNA to ribosomes are described.

## Materials and Methods

*Paper Chromatography and Electrophoresis.* The following solvents were employed for purification or identification of labeled and unlabeled oligonucleotides: solvent A, 1-propanol–ammonium hydroxide–$H_2O$ (55:10:35, v/v) (Jones *et al.*, 1964); solvent B, 400 g of ammonium sulfate dissolved in 1.0 l. of 1.0 M sodium phosphate (pH 7.0) (Rushizky and Knight, 1960); solvent C, 95% ethanol–1.0 M ammonium acetate (Thach and Sundararajan, 1965); and solvent D, 0.2 M ammonium formate (Bernfield, 1966). Whatman No. 3MM filter paper was used with solvents A, B, and C and Whatman DEAE filter paper (DE81) with solvent D. Electrophoresis was performed with 0.05 M ammonium formate (pH 2.7) using 4500 V for 40 min on Whatman No. 3MM filter paper for purification and identification of oligonucleotides of chain length 5 or less.

*Preparation of Oligonucleotides.* [5-$^3$H]Cytidine, [5-$^3$H]uridine, and uniformly labeled [$^3$H]ADP and [$^3$H]UDP with specific radioactivities of 6.0, 2.3, 1.6, and 3.2 Ci per mmole, respectively, were purchased from Schwarz BioResearch Co.

[$^3$H]UC, [$^3$H]UUC, and [$^3$H]UUUC were synthesized with [$^3$H]cytidine as the labeled moiety and [$^3$H]UU and [$^3$H]UUU were synthesized with [$^3$H]uridine as the labeled moiety by the same procedure used in preparing the corresponding unlabeled oligonucleotides (Bernfield, 1966) in collaboration with M. R. Bernfield. [$^3$H]ADP was polymerized with polynucleotide phosphorylase as previously described (Singer and Guss, 1962). Nonprimer requiring polynucleotide phosphorylase from *Micrococcus lysodeikticus* was the gift of P. Leder; primer-dependent polynucleotide phosphorylase was the gift of P. Leder and F. Anderson. The resulting [$^3$H]poly(A) was digested with micrococcal nuclease (Alexander *et al.*, 1961) that was free of phosphomonoesterase activity (Worthington Biochemical) and [$^3$H]AAp and [$^3$H]AAAp were obtained as products. The terminal 3-phosphate was removed from a portion of each with *Escherichia coli* alkaline phosphatase free of diesterase activity (Worthington Biochemical, chromatographically purified) as previously described (Rottman and Nirenberg, 1966). Only [$^3$H]AA, [$^3$H]AAA, and [$^3$H]AAAp were used in the present study.

[$^3$H]UDP was polymerized with primer-requiring polynucleotide phosphorylase and AC by the procedure of Leder *et al.* (1965) as modified by F. Anderson (unpublished results). [$^3$H]Oligonucleotides of chain length 3–7 were purified after polymerization from the reaction mixture; [$^3$H]$ACU_{1-5}$ preparations were prepared with F. Anderson. Specific radioactivities of [$^3$H]oligonucleotide ranged from 200 to 550 cpm per $\mu\mu$mole at a counting efficiency of 10%.

*tRNA Preparations.* Uniformly labeled L-[$^{14}$C]phenylalanine (282 mCi/mmole), L-[$^{14}$C]serine (120 mCi/mmole), L-[$^{14}$C]lysine (222 mCi/mmole), L-[$^{14}$C]threonine (160 mCi/mole), and L-[$^{14}$C]leucine (222 mCi/mmole) were obtained from New England Nuclear Corp. or Nuclear-Chicago Corp. [$^{14}$C]Aminoacyl-tRNA was prepared with one labeled amino acid, 19 unlabeled amino acids, tRNA from *E. coli* W3100 (the gift of S. Pestka), or with tRNA from *E. coli* B (General Biochemicals Co.) and *E. coli* W3100 100,000*g* supernatant fraction. Twenty unlabeled amino acids were used to prepare [$^{12}$C]aa-tRNA.

*Reaction Mixture Components and Assays.* Three-times-washed ribosomes (Nirenberg, 1963) from *E. coli* W3100 (the gift of S. Pestka) were used in assays with [$^3$H]UUC; ribosomes used with other oligonucleotides were prepared (first washing step) in the presence of 20% $(NH_4)_2SO_4$.

Unless otherwise indicated, reaction mixtures were incubated for 5 min at 3° prior to incubation at 24°. Reactions contained the following components (unless otherwise stated) in a final volume of 0.05 ml: standard buffer [0.1 M Tris–acetate (pH 7.2)–0.03 M magnesium acetate–0.05 M potassium acetate], [$^3$H]- or [$^{12}$C]UUC (2.33 m$\mu$moles), and *E. coli* ribosomes (1.80 $A_{260}$ units). Reactions were initiated by addition

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[1] Abbreviations used are: aa, aminoacyl; AAA and AAAp correspond to ApApA (without terminal phosphate) and ApApAp (with 3′-terminal phosphate), respectively; $ACU_2$, $ACU_3$, etc., correspond to ApCpUpU, ApCpUpUpU.

TABLE I: Characterisrics of Binding.[a]

| Modification | Additions | μμmoles Bound to Ribosomes: [³H]-UUC | μμmoles Bound to Ribosomes: [¹⁴C]Phe-tRNA |
|---|---|---|---|
| Minus Ribosomes | [³H]UUC + [¹⁴C]Phe-tRNA | 0.43 | 0.03 |
| Minus $Mg^{2+}$ | [³H]UUC + [¹⁴C]Phe-tRNA | 0.52 | 0.12 |
| Minus tRNA | [³H]UUC | 1.10 | |
| Complete | [³H]UUC + [¹²C]Phe-tRNA | 2.63 | |
| Complete | [³H]UUC + [¹⁴C]Phe-tRNA | 2.39 | 1.45 |
| Complete | [¹²C]UUC + [¹⁴C]Phe-tRNA | | 1.45 |

[a] Each reaction contained the components described under Methods except where indicated and the following: 2.33 mμmoles of [³H]UUC or 2.28 mμmoles of unlabeled UUC; and 9.56 μμmoles of [¹⁴C]Phe-tRNA (0.30 $A_{260}$ unit) or 0.31 $A_{260}$ unit of aa-tRNA.

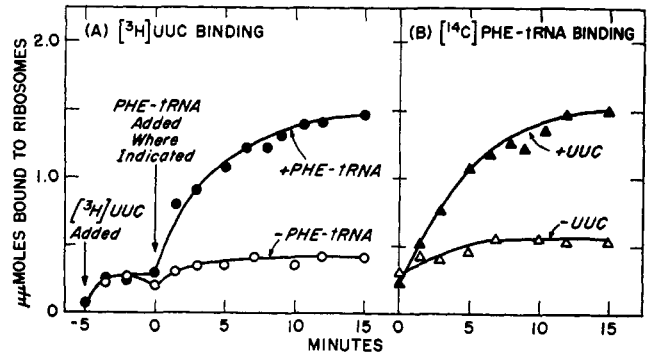


FIGURE 1: (A) The effect of Phe-tRNA on the rate of [³H]UUC binding to ribosomes. (B) The effect of UUC on the rate of [¹⁴C]-Phe-tRNA binding to ribosomes. The symbols in part A represent the following: (○) minus aa-tRNA; (●) 9.5 μμmoles of labeled Phe-tRNA (0.30 $A_{260}$ unit) per 50-μl reaction added at zero time. Each reaction contained [³H]UUC and the components described under Methods. Incubation was at 3° from −5 to 0 min and at 24° from 0 to 15 min. The symbols in part B represent the following: (△) minus UUC; (▲) plus [³H]UUC. Each 50-μl reaction contained 9.5 μμmoles of [¹⁴C]Phe-tRNA (0.30 $A_{260}$ unit) and the components described under Methods. Incubation was at 24°. Each point represents a 50-μl portion removed from a larger reaction at the time stated.

of [¹⁴C]- or [¹²C]Phe-tRNA, as specified in the legend accompanying each table or figure, or by addition of an equal volume of water. Components and conditions of reaction mixtures used in experiments with other [³H]oligonucleotides are described in the legends. Ribosomal-bound [³H]oligonucleotides or [¹⁴C]aa-tRNAs were washed on Millipore filters as described previously (Nirenberg and Leder, 1964) except that five, 3-ml portions and then two, 5-ml portions of standard buffer at 3° were used. This modification was necessary to reduce nonspecific interactions between [³H]oligonucleotides and Millipore filters.

## Results

*Binding of [³H]UUC and [¹⁴C]Phe-tRNA to Ribosomes.* The effect of aa-tRNA upon the rate of [³H]UUC binding to ribosomes is shown in Figure 1A. A small amount of [³H]UUC attached to ribosomes at 3° (prior to zero time shown in Figure 1A); at 24° further attachment of UUC to ribosomes was dependent upon aa-tRNA. As shown in Figure 1B, about 0.5 μμmole of [¹⁴C]Phe-tRNA bound to ribosomes in the absence of UUC. The addition of UUC markedly stimulated [¹⁴C]-Phe-tRNA binding. These data show that [³H]UUC and [¹⁴C]-Phe-tRNA bind to ribosomes at similar rates and to approximately the same extent.

Some control experiments are shown in Table I. In the absence of ribosomes or $Mg^{2+}$, cellulose–nitrate filters retain little [³H]UUC and [¹⁴C]Phe-tRNA. Retention of UUC by filters was dependent upon ribosomes, Phe-tRNA and $Mg^{2+}$; similarly, retention of Phe-tRNA was dependent upon ribosomes, UUC, and $Mg^{2+}$. The assay for [³H]oligonucleotide binding was highly reproducible, both with single- and double-label components.

The relation between $Mg^{2+}$ concentration and binding of [³H]UUC and [¹⁴C]Phe-tRNA to ribosomes is shown in Figure 2A,B, respectively. Phe-tRNA-dependent binding of [³H]UUC was observed between 0.02 and 0.10 M $Mg^{2+}$. Similar results were obtained for UUC-dependent Phe-tRNA binding although less [¹⁴C]Phe-tRNA bound to ribosomes than UUC. Maximal binding was found at 0.03–0.04 M $Mg^{2+}$.

Phe-tRNA stimulated the binding of [³H]UUC to ribosomes at each UUC concentration investigated. In most experiments 2.3–2.5 mμmoles of [³H]UUC was added to each 50-μl reaction to conserve radioactive trinucleotides. Approximately 0.150 $A_{260}$ unit of aa-tRNA was required for maximal binding of [³H]UUC to ribosomes. Binding of [¹⁴C]Phe-tRNA was almost maximal with 4 mμmoles of UUC. Maximal [¹⁴C]-Phe-tRNA binding in response to UUC occurred with 4 μμmoles of Phe-tRNA.

*Effect of tRNA and aa-tRNA on the Binding of [³H]UUC to Ribosomes.* The binding of tRNA in response to oligonucleotides has been reported by Levin (1970). Greater attachment of [³H]UUC to ribosomes was observed in the presence of aa-tRNA than in the presence of tRNA at 0.03 M $Mg^{2+}$ and limiting tRNA concentrations.

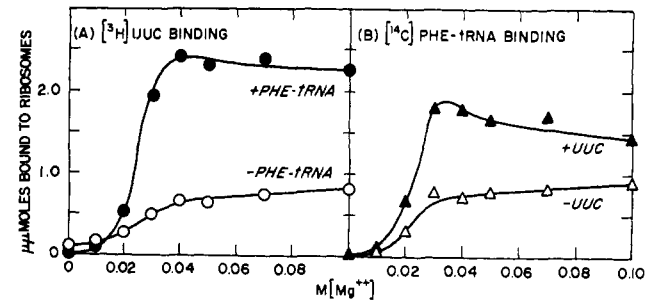


FIGURE 2: Relation between $Mg^{2+}$ and (A) [³H]UUC binding to ribosomes, ±Phe-tRNA; and (B) [¹⁴C]Phe-tRNA binding to ribosomes, ±UUC. Each symbol represents a 50-μl reaction containing the components described under Methods and in (A) [³H]UUC and the following: (●) 0.29 $A_{260}$ unit of unlabeled aa-tRNA; (○) minus aa-tRNA. In part B each reaction contained 8.94 μμmoles of [¹⁴C]-Phe-tRNA (0.31 $A_{260}$ unit) and the following: (▲) 2.28 mμmoles of unlabeled UUC; (△) minus UUC. Reactions were washed onto Millipore filters with standard buffers containing $Mg^{2+}$ concentrations stated in the figure.

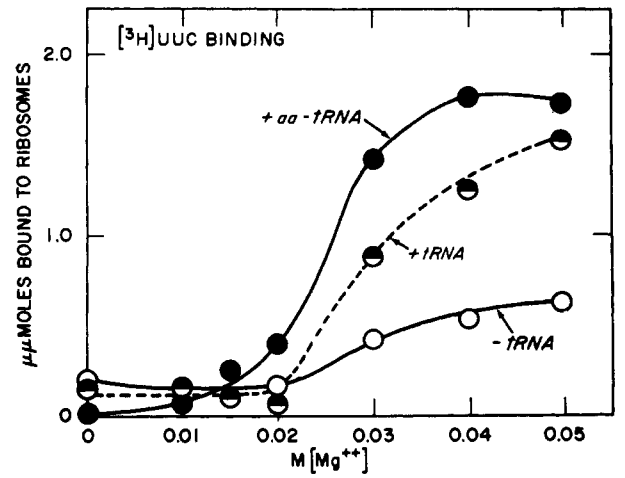


FIGURE 3: Binding of [$^3$H]UUC to ribosomes in the presence of the following: (●) aa-tRNA, (◓) tRNA, (○) minus tRNA at the $Mg^{2+}$ concentrations indicated. Each point represents a 50-$\mu$l reaction containing the components described under Methods, [$^3$H]UUC, and where indicated, 0.11 $A_{260}$ unit of unlabeled aa-tRNA or 0.12 $A_{260}$ unit of tRNA, prepared as follows. *E. coli* W 3100 tRNA was placed into a reaction containing each of the components required for tRNA acylation. Phenol was added immediately to prevent acylation and tRNA was recovered.

In Figure 3 the effect of $Mg^{2+}$ concentration upon the binding of [$^3$H]UUC in the presence of acylated and deacylated tRNA and in the absence of tRNA is shown. Appreciable binding of [$^3$H]UUC to ribosomes was observed only in reactions containing greater than 0.02 M $Mg^{2+}$. At 0.02–0.04 M $Mg^{2+}$, aa-tRNA stimulated more [$^3$H]UUC binding to ribosomes

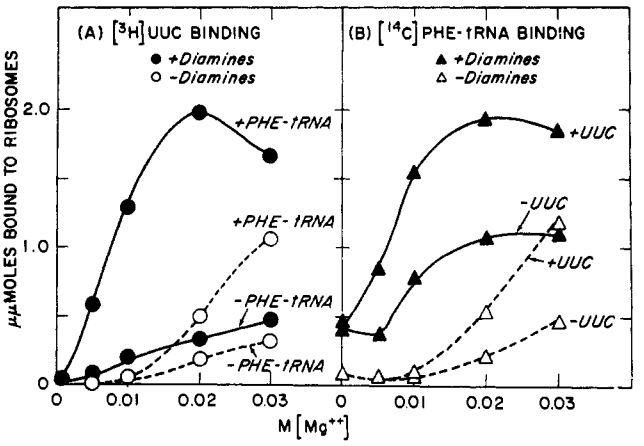


FIGURE 4: The effect of diamines on (A) [$^3$H]UUC and (B) [$^{14}$C]-Phe-tRNA binding to ribosomes in relation to $Mg^{2+}$ concentration. Each symbol represents a 50-$\mu$l reaction containing the components described under Methods, 1.55 $A_{260}$ units of ribosomes and in (A) [$^3$H]UUC and the following: (●) 0.013 M putrescine and 0.002 M spermidine; (○) minus diamines. [$^{14}$C]Phe-tRNA (7.76 $\mu\mu$moles, 0.24 $A_{260}$ unit) where indicated. In part B each reaction contained the components described under Methods, 7.76 $\mu\mu$moles of [$^{14}$C]Phe-tRNA (0.24 $A_{260}$ unit) and the following: (▲) 0.013 M putrescine and 0.002 M spermidine; (△) minus diamines. Labeled UUC was added where indicated. The order of addition of each component to reactions and time and temperature of incubation were as follows. Ribosomes in the presence of standard buffer and in the presence or absence of diamines were incubated for 10 min at 3°; then [$^3$H]UUC was added where indicated and reactions were incubated for an additional 5 min at 3°. [$^{14}$C]Phe-tRNA was added where indicated and reactions were incubated for 15 min at 24°.

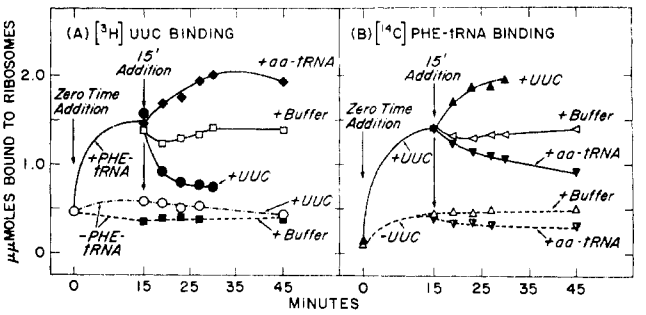


FIGURE 5: (A) The effect of UUC and aa-tRNA on ribosomal-bound [$^3$H]UUC and (B) the effect of UUC and aa-tRNA on ribosomal-bound [$^{14}$]Phe-tRNA. The additions and symbols represent the following. (A) At zero time: ○-, 6.66 $\mu\mu$moles of labeled Phe-tRNA (0.21 $A_{260}$ unit) per 50-$\mu$l reaction; and ○···, and ○- - -, minus aa-tRNA. At 15 min in reactions with Phe-tRNA; (◆) 1.02 $A_{260}$ units of unlabeled aa-tRNA, (□) buffer, and (●) 11.5 m$\mu$moles of unlabeled UUC. At 15 min in reactions without Phe-tRNA: (○) 11.5 m$\mu$moles of unlabeled UUC, and (■) buffer. Each 50-$\mu$l reaction contained the components described under Methods and [$^3$H]-UUC. Additions at 15 min contained, in a 5-$\mu$l volume, standard buffer (see Methods) or standard buffer with unlabeled UUC or aa-tRNA. In part B at zero time: 6.66 $\mu\mu$moles of [$^{14}$C]Phe-tRNA (0.21 $A_{260}$ unit) per 50-$\mu$l reaction, (▲) labeled UUC, and (△) minus UUC. At 15 min in reactions with UUC: (▼) 1.02 $A_{260}$ units of unlabeled aa-tRNA, (◁) buffer, and (▲) 11.5 m$\mu$moles of unlabeled UUC. At 15 min in reactions without UUC: (△) 1.02 $A_{260}$ units of unlabeled aa-tRNA and (▽) buffer. Each 50-$\mu$l reaction contained the components described under Methods and 6.66 $\mu\mu$moles of [$^{14}$C]Phe-tRNA. Additions at 15 min contained the components given above in part A.

than did deacylated tRNA; and at 0.05 M $Mg^{2+}$ only slightly more [$^3$H]UUC binding to ribosomes occurred in the presence of aa-tRNA than in the presence of deacylated tRNA.

*Effect of Putrescine and Spermidine.* The effect of the diamines, putrescine and spermidine, on the binding of [$^3$H]UUC and [$^{14}$C]Phe-tRNA to ribosomes, in relation to $Mg^{2+}$ concentration, is shown in Figure 4A,B. In these experiments, 0.013 M putrescine and 0.002 M spermidine were used. In the absence of Phe-tRNA, the diamines had little effect on binding of [$^3$H]UUC to ribosomes. However, in the presence of Phe-tRNA, the diamines greatly enhanced binding of [$^3$H]-UUC. The effect of putrescine and spermidine on binding of [$^{14}$C]Phe-tRNA to ribosomes is shown in Figure 4B. The diamines stimulated the binding of [$^{14}$C]Phe-tRNA, even in the absence of UCC. The increased binding of [$^{14}$C]Phe-tRNA due to the diamines in the absence of UUC is not dependent upon template, since binding of [$^3$H]UUC is stimulated only slightly by diamines in the absence of Phe-tRNA. Therefore, diamines appear to have a greater effect upon aa-tRNA–ribosome interactions.

*Oligonucleotide Chain Length Studies.* In Table II, the binding of the dinucleotides, [$^3$H]UU, [$^3$H]UC, and [$^3$H]AA, the trinucleotides, [$^3$H]UUC, [$^3$H]AAA, and [$^3$H]AAAp, and the tetranucleotide, [$^3$H]UUUC, is shown. In expt 1, little or no binding of [$^3$H]UU or [$^3$H]UC was observed either in the presence or absence of Phe-tRNA. Also, the dinucleotides did not stimulate the binding of [$^{14}$C]Phe-tRNA to ribosomes.

The binding of [$^3$H]UUC and [$^3$H]UUU to ribosomes in the presence and absence of [$^{14}$C]Phe-tRNA is also shown in expt 1. The binding of both trinucleotides to ribosomes was stimulated in the presence of Phe-tRNA. Both trinucleotides stimulated the attachment of [$^{14}$C]Phe-tRNA to ribosomes; however, [$^3$H]UUC is a more effective template for [$^{14}$C]Phe-tRNA than [$^3$H]UUU. The binding of the tetranucleotide, [$^3$H]-

TABLE II: Binding of [$^3$H]Oligonucleotides of Varying Chain Length to Ribosomes.[a]

| Expt | Additions | μμmoles of [$^3$H]Oligonucleotide Bound to Ribosomes | | | μμmoles of [$^{14}$C]aa-tRNA Bound to Ribosomes | | |
|---|---|---|---|---|---|---|---|
| | | −tRNA | +aa-tRNA | Δ | −Oligo | +Oligo | Δ |
| 1 | [$^3$H]UU and [$^{14}$C]Phe-tRNA | 0.10 | 0.04 | −0.06 | 0.69 | 0.62 | −0.07 |
| | [$^3$H]UC and [$^{14}$C]Phe-tRNA | 0.10 | 0.07 | −0.03 | 0.69 | 0.56 | −0.13 |
| | [$^3$H]UUU and [$^{14}$C]Phe-tRNA | 0.54 | 0.99 | 0.45 | 0.69 | 1.12 | 0.43 |
| | [$^3$H]UUC and [$^{14}$C]Phe-tRNA | 0.51 | 2.06 | 1.55 | 0.69 | 1.84 | 1.15 |
| | [$^3$H]UUUC and [$^{14}$C]Phe-tRNA | 0.86 | 3.20 | 2.34 | 0.69 | 2.17 | 1.48 |
| 2 | [$^3$H]AA and [$^{14}$C]Lys-tRNA | 0.22 | 0.19 | −0.03 | 0.31 | 0.36 | 0.05 |
| 3 | [$^3$H]AAA and [$^{14}$C]Lys-tRNA | 0.51 | 1.82 | 1.31 | 0.22 | 1.66 | 1.44 |
| | [$^3$H]AAAp and [$^{14}$C]Lys-tRNA | 0.71 | 1.36 | 0.65 | 0.22 | 1.11 | 0.89 |
| 4 | [$^3$H]ACU and [$^{14}$C]Thr-tRNA | 0.81 | 2.72 | 1.91 | 0.27 | 1.53 | 1.26 |
| | [$^3$H]$ACU_2$ and [$^{14}$C]Thr-tRNA | 2.42 | 3.68 | 1.26 | 0.27 | 0.72 | 0.45 |
| | [$^3$H]$ACU_3$ and [$^{14}$C]Thr-tRNA | 1.18 | 4.09 | 2.91 | 0.27 | 0.54 | 0.27 |
| | [$^3$H]$ACU_4$ and [$^{14}$C]Thr-tRNA | 0.57 | 4.04 | 3.47 | 0.27 | 0.47 | 0.20 |
| | [$^3$H]$ACU_5$ and [$^{14}$C]Thr-tRNA | 0.68 | 4.90 | 4.22 | 0.27 | 0.48 | 0.21 |
| | [$^3$H]$ACU_2$ and [$^{14}$C]Leu-tRNA | 2.42 | 3.68 | 1.26 | 1.65 | 1.62 | −0.03 |
| | [$^3$H]$ACU_3$ and [$^{14}$C]Leu-tRNA | 1.18 | 4.24 | 3.06 | 1.65 | 1.44 | −0.21 |
| | [$^3$H]$ACU_4$ and [$^{14}$C]Leu-tRNA | 0.57 | 4.19 | 3.62 | 1.65 | 1.19 | −0.46 |
| | [$^3$H]$ACU_5$ and [$^{14}$C]Leu-tRNA | 0.68 | 4.53 | 3.85 | 1.65 | 1.34 | −0.31 |
| | [$^3$H]$ACU_3$ and [$^{14}$C]Phe-tRNA | 1.18 | 3.89 | 2.71 | 0.43 | 1.66 | 1.23 |
| | [$^3$H]$ACU_4$ and [$^{14}$C]Phe-tRNA | 0.57 | 3.50 | 2.93 | 0.43 | 1.94 | 1.51 |
| | [$^3$H]$ACU_5$ and [$^{14}$C]Phe-tRNA | 0.68 | 4.18 | 3.50 | 0.43 | 2.16 | 1.73 |

[a] In expt 1–3, each reaction contained the components described under Methods and two additional components, one labeled for expt 1, the other labeled as specified. Labeled components were: [$^3$H]UU, 2.34 mμmoles; [$^3$H]UC, 2.36 mμmoles; [$^3$H]UUU, 2.33 mμmoles; [$^3$H]UUC, 2.36 mμmoles; [$^3$H]UUUC, 2.58 mμmoles; and [$^{14}$C]Phe-tRNA, 9.56 μμmoles (0.30 $A_{260}$ unit); unlabeled components were: UU, 2.58 mμmoles; UC, 2.40 mμmoles; UUU, 2.50 mμmoles, UUC, 2.28 mμmoles; UUUC, 2.43 mμmoles; and 0.28 $A_{260}$ unit of aa-tRNA. In expt 2, each reaction contained in a 50-μl volume, 0.1 M Tris–acetate (pH 7.2), 0.05 M potassium acetate, 0.02 M magnesium acetate, 1.19 $A_{260}$ units of ribosomes, and the following. Labeled components: [$^3$H]AA, 1.22 mμmole; and [$^{14}$C]Lys-tRNA, 7.1 μμmoles (0.16 $A_{260}$ unit). Unlabeled components: AA, 1.11 mμmoles; and aa-tRNA (0.15 $A_{260}$ unit). In expt 3 each reaction contained the components described in expt 2 with the following exceptions. 0.53 $A_{260}$ unit of ribosomes washed with 20% $(NH_4)_2SO_4$ to remove tRNA as described under Methods; and labeled components: [$^3$H]AAA, 0.84 mμmole; [$^3$H]AAAp, 0.82 mμmole; and [$^{14}$C]Lys-tRNA, 9.1 μμmoles (0.22 $A_{260}$ unit). Unlabeled components: AAA, 0.86 mμmole; AAAp, 0.82 mμmole; and aa-tRNA (0.22 $A_{260}$ unit). In expt 4, each reaction contained the components described in expt 2 and the following: [$^3$H]ACU, 2.61 mμmoles; [$^3$H]$ACU_2$, 2.44 mμmoles; [$^3$H]$ACU_3$, 2.42 mμmoles; [$^3$H]$ACU_4$, 2.27 mμmoles; [$^3$H]$ACU_5$, 2.30 mμmoles; [$^{14}$C]Thr-tRNA, 10.4 μμmoles (0.20 $A_{260}$ unit); [$^{14}$C]Leu-tRNA, 18.5 μμmoles (0.20 $A_{260}$ unit); [$^{14}$C]Phe-tRNA, 5.3 μμmoles (0.19 $A_{260}$ unit); and 0.96 $A_{260}$ unit of ribosomes.

UUUC, to ribosomes was greater than either trinucleotide; the tetranucleotide also was a more active template for [$^{14}$C]-Phe-tRNA binding than the trinucleotides.

In expt 2 and 3, the binding of [$^3$H]AA, [$^3$H]AAA, and [$^3$H]-AAAp to ribosomes is shown. Little binding of [$^3$H]AA to ribosomes was observed with or without Lys-tRNA. Similarly, AA did not stimulate the binding of [$^{14}$C]Lys-tRNA. Addition of aa-tRNA resulted in a slight increase in attachment of [$^3$H]AAA and [$^3$H]AAAp to ribosomes; however, both trinucleotides markedly stimulated the binding of [$^{14}$C]Lys-tRNA (data not shown). Since a small contamination of ribosomes with $tRNA^{Lys}$ might be responsible for the binding of [$^3$H]AAA and [$^3$H]AAAp in the absence of added Lys-tRNA, binding to ribosomes of higher purity was studied (expt 3). Both [$^3$H]AAA and [$^3$H]AAAp stimulated the attachment of [$^{14}$C]Lys-tRNA. [$^3$H]AAA bound to ribosomes slightly more effectively than [$^3$H]AAAp in the presence of Lys-tRNA. Similarly, AAA stimulated [$^{14}$C]Lys-tRNA binding to a greater extent than AAAp.

The binding to ribosomes of a series of oligonucleotides of chain length 3–7 and of [$^{14}$C]aa-tRNA is shown in expt 4 of Table II. The binding of each [$^3$H]oligonucleotide preparation was markedly stimulated by the presence of aa-tRNA. Oligonucleotides containing $ACU_{1-5}$ stimulated Thr- or Phe-tRNA binding to ribosomes, but not Leu-tRNA. As shown previously, the trinucleotide, CUU, has little effect upon Leu-tRNA binding (Bernfield and Nirenberg, 1965), although polynucleotides containing CUU sequences actively stimulate leucine incorporation into protein (Khorana *et al.*, 1966). The attachment of [$^{14}$C]Thr-tRNA to ribosomes was considerably greater with ACU than with oligonucleotides of longer chain length.

*Effect of Unlabeled UUC and aa-tRNA on Ribosomal-Bound [$^3$H]UUC and [$^{14}$C]Phe-tRNA.* The rates of exchange of ribosomal-bound [$^3$H]UUC and [$^{14}$C]Phe-tRNA with free, unlabeled UUC and aa-tRNA, respectively, are shown in Figure 5A,B. Reactions were incubated 15 min at 24° with or without added Phe-tRNA to permit binding of [$^3$H]UUC to ribosomes (Figure A); then, either unlabeled UUC, aa-tRNA or buffer was added. The addition of a 5-fold excess of unlabeled UUC

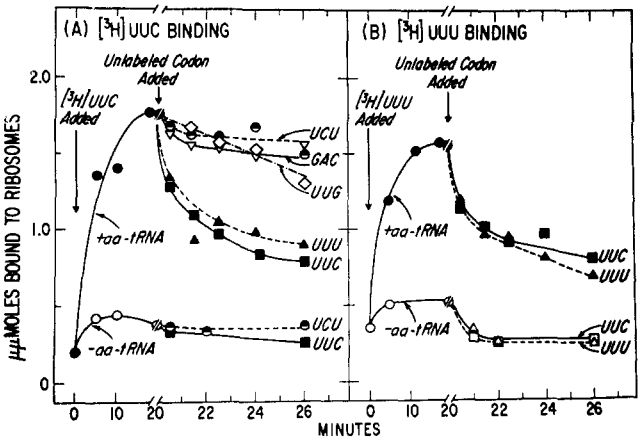


FIGURE 6: The effect of unlabeled codons on ribosomal-bound (A) [$^3$H]UUC and (B) [$^3$H]UUU. The additions and symbols represent the following. (A) At zero time: (●) 0.27 $A_{260}$ unit of unlabeled aa-tRNA/50-$\mu$l reaction mixture and (○) minus aa-tRNA. At 15 min in reactions with aa-tRNA: (◓) 12.19 m$\mu$moles of UCU, (▽) 9.91 m$\mu$moles of GAC, (◇) 16.29 m$\mu$moles of UUG, (▲) 11.93 m$\mu$moles of UUU, and (■) 11.54 m$\mu$moles of UUC. At 15 min in reactions without aa-tRNA: (◓) 12.19 m$\mu$moles of UCU and (■) 11.54 m$\mu$moles of UUC. Each 50-$\mu$l reaction contained the components described under Methods, 1.25 $A_{260}$ units of ribosomes and 2.97 m$\mu$moles of [$^3$H]UUC. Additions at 15 min contained, in a 5-$\mu$l volume, unlabeled codon and standard buffer. In part B, at zero time: (●) 0.27 $A_{260}$ unit of unlabeled aa-tRNA/50-$\mu$l reaction; and (○) minus aa-tRNA. At 15 min in reactions with aa-tRNA: (■) 11.54 m$\mu$moles of UUC; and (▲) 11.93 m$\mu$moles of UUU. At 15 min in reactions without aa-tRNA: (□) 11.54 m$\mu$moles of UUC; and (△) 11.93 m$\mu$moles of UUU. Each 50-$\mu$l reaction contained the components described in part A and 2.77 m$\mu$moles of [$^3$H]UUU. Additions at 15 min contained, in a 5-$\mu$l volume, unlabeled codon and standard buffer.

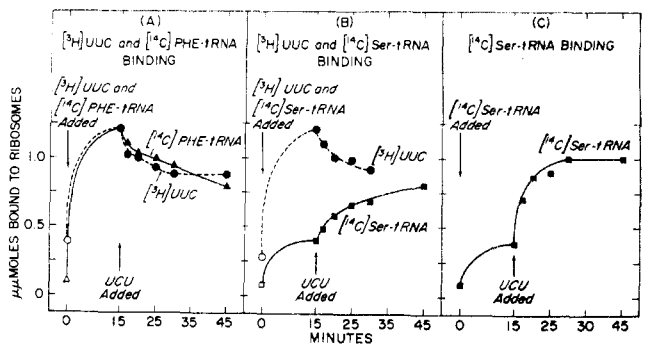


FIGURE 7: The effect of UCU on (A) ribosomal-bound [$^3$H]UUC and [$^{14}$C]Phe-tRNA, (B) ribosomal-bound [$^3$H]UUC and the binding of [$^{14}$C]Ser-tRNA to ribosomes, and (C) the binding of [$^{14}$C]Ser-tRNA to ribosomes. The additions and symbols represent the following: (A) at zero time, (△) 7.14 m$\mu$moles of [$^{14}$C]Phe-tRNA (plus 19 unlabeled aa-tRNAs, 0.20 $A_{260}$ unit) and (○) [$^3$H]UUC. The [$^3$H]UUC bound at zero time represents that binding which occurred during a 5-min preincubation period at 3°. At 15 min, 7.22 m$\mu$moles of UCU in a 5-$\mu$l volume of standard buffer were added and the displacement of [$^{14}$C]Phe-tRNA (▲) and of [$^3$H]UUC (●) were assayed at the time intervals shown. After 15 min at 24°, 0.48 $\mu\mu$mole of [$^{14}$C]Phe-tRNA bound to ribosomes and 0.38 $\mu\mu$mole of [$^3$H]UUC bound to ribosomes in reactions minus aa-tRNA (data not shown). In part B at zero time (□), 7.49 $\mu\mu$moles of [$^{14}$C]Ser-tRNA (plus 19 unlabeled aa-tRNAs, 0.21 $A_{260}$ unit) and (○) [$^3$H]UUC. At 15 min, 7.22 m$\mu$moles of UCU was added as above and the displacement of ribosomal-bound [$^3$H]UUC (●), and the binding of [$^{14}$C]Ser-tRNA (■) were assayed at the time intervals shown. In part C at zero time, 7.49 $\mu\mu$moles of [$^{14}$C]Ser-tRNA was added; and at 15 min, 7.22 m$\mu$moles of UCU was added.

resulted in a rapid release of [$^3$H]UUC from ribosomes; the addition of aa-tRNA stimulated further attachment of [$^3$H]-UCC to ribosomes; the addition of buffer had little effect. In Figure 5B, corollary experiments with [$^{14}$C]Phe-tRNA are shown. Ribosomal-bound [$^{14}$C]Phe-tRNA was slowly released from the ribosomes upon addition of a 5-fold excess of unlabeled aa-tRNA. Addition of UUC stimulated further attachment of [$^{14}$C]Phe-tRNA to ribosomes; whereas, addition of buffer had little effect. [$^{14}$C]Phe-tRNA that bound to ribosomes in the absence of UUC was only slightly affected by the addition of aa-tRNA. The results show that UUC is released from ribosomes more rapidly than Phe-tRNA, and that the binding equilibria shifts upon further addition of UUC or Phe-tRNA.

*Effect of Unlabeled Trinucleotides on Ribosomal-Bound [$^3$H]UUC, [$^3$H]UUU, and [$^{14}$C]Phe-tRNA.* In Figure 6, the effect of a 5-fold excess of unlabeled UUC, UUU, UCU, GAC, or UUG upon ribosomal-bound [$^3$H]UUC or [$^3$H]UUU is shown. Marked differences were observed between the initial rates of [$^3$H]UUC displacement from ribosomes due to the addition of unlabeled trinucleotides. The initial rate of displacement of ribosomal-bound [$^3$H]UUC was more rapid in the presence of Phe–codon, UUC or UUU, than in the presence of codons for other amino acids (GAC, UUG, and UCU). [$^3$H]UUU was displaced from codon recognition sites at approximately the same rate as [$^3$H]UUC in presence of UUU or UUC (Figure 6B). These data must be viewed in context with the data in Figure 5, which demonstrate that ribosomal-bound [$^3$H]UUC is released more rapidly than [$^{14}$C]-Phe-tRNA from codon–ribosome–aa-tRNA complexes. The data strongly suggest that the specificity of trinucleotide exchange is directed by the anticodon of Phe-tRNA that remains bound to ribosomes.

The effect of the Ser–codon, UCU, on the release of [$^3$H]-UUC and [$^{14}$C]Phe-tRNA from ribosomes and on the binding of [$^{14}$C]Ser-tRNA to ribosomes is shown in Figure 7A, B, and C, respectively. [$^3$H]UUC, [$^{14}$C]Phe-tRNA, and ribosomes were incubated to form the [$^3$H]UUC–ribosome–[$^{14}$C]-Phe-tRNA complexes, then a 3-fold excess of UCU was added. [$^3$H]UUC and [$^{14}$C]Phe-tRNA were released from ribosomes slowly and at similar rates (Figure 7A). When [$^3$H]UUC and [$^{12}$C]Phe-tRNA were permitted to bind to ribosomes but in the presence of [$^{14}$C]Ser-tRNA, and again a 3-fold excess of UCU added at 15 min, a decrease in ribosomal-bound [$^3$H]-UUC and an increase in bound [$^{14}$C]Ser-tRNA were observed (Figure 7B). A considerably more rapid binding of [$^{14}$C]Ser-tRNA to ribosomes occurred in response to UCU addition at 15 min, when [$^3$H]UUC was not present in the reaction (Figure 7C). These data show that [$^3$H]UUC and [$^{14}$C]Phe-tRNA were released from ribosomes at similar rates due to addition of the Ser–codon, UCU, and that the rate of [$^{14}$C]-Ser-tRNA binding to ribosomes in response to UCU was reduced when ribosomal binding sites are occupied by codons and aa-tRNA for another amino acid.

## Discussion

The results show that the binding of [$^3$H]oligonucleotides to ribosomes is dependent upon aa-tRNA. Under the conditions studied, binding of both trinucleotide and aa-tRNA to ribosomes is reversible; hence the amount of trinucleotide–ribosome–aa-tRNA complex measured represents the sum of both association and dissociation of the ribosomal complex. The stability of the ribosomal complex depends upon many

factors, including interactions between mRNA–codon and tRNA–anticodon, tRNA and ribosome, and trinucleotide and ribosome.

Some codons such as AAA, bound to ribosomes to a greater extent than others, such as UUC. Even in the case of the synonym codons, UUU and UUC, differences in the extent of codon–ribosome–aa-tRNA complex formation were observed.

Ribosomal-bound UUC and Phe-tRNA were found to exchange with unbound UUC and Phe-tRNA. The rate of exchange of bound with unbound UUC was more rapid than the exchange of bound with unbound Phe-tRNA. Ribosomal-bound [$^3$H]UUC exchanged more rapidly with an unbound *synonym* codon, UUU, than with codons for leucyl-, seryl-, and aspartyl-tRNA, respectively.

These results strongly suggest that the anticodon of ribosomal-bound aa-tRNA can act as a template for a *set* of synonym trinucleotides during the exchange process much as a trinucleotide acts as a template for aa-tRNA. In this way, the specificity of a species of aa-tRNA for a set of codons can be elucidated with unfractionated tRNA by determining the rate of release of a radioactive trinucleotide from the trinucleotide–ribosome–aa-tRNA complex in the presence of unlabeled synonym triplets.

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# Reaction of 7-Bromomethylbenz[*a*]anthracene with Nucleic Acids, Polynucleotides, and Nucleosides*

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ABSTRACT: In order to investigate the mode of action of the carcinogen 7-bromomethylbenz[*a*]anthracene, the chemistry of its reaction with nucleic acids and their constituents has been studied. It appears that in dimethylacetamide this reagent reacts with nucleosides mainly at the same position as do methylating agents *i.e.*, N-7 of guanine derivatives, N-1 of adenine derivatives, and N-3 of cytosine derivatives. However, in aqueous solution, reaction with nucleosides, nucleic acids, or polynucleotides leads mainly to reaction on the amino groups of guanine, adenine, and possibly cytosine.

In earlier studies 7-bromomethylbenz[*a*]anthracene did not induce sarcoma in the rat, and was thereby shown to be a less active carcinogen than its parent hydrocarbon, 7-methylbenz[*a*]anthracene (Dipple and Slade, 1970). However, at a higher dose, tumors are produced in the rat (A. Dipple and T. A. Slade, unpublished data), and this bromo compound is also known to be active in the initiation of papilloma in mouse skin (J. D. Scribner, personal communication; Dipple and Slade, 1971). An understanding of the chemistry of the reaction of this agent with cellular constituents could obviously clarify the mechanism by which it evokes the carcinogenic response and could conceivably, though not necessarily, cast some light on the mechanism of action of the aromatic hydrocarbons themselves.

Earlier studies (Brookes and Lawley, 1964) have suggested that DNA may be the critical receptor for the aromatic hydrocarbon carcinogens, and for this reason we have studied in the first instance the reactions of this new chemical carcinogen with nucleic acids.

Brookes and Dipple (1969) presented preliminary data

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