Role of Modified Nucleosides in Transfer Ribonucleic Acid

EFFECT OF REMOVAL OF THE MODIFIED BASE ADJACENT TO 3' END OF THE ANTICODON IN CODON-ANTICODON INTERACTION*

KAKOLI GHOSH AND HARA PRASAD GHOSH†

From the Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada

SUMMARY

Removal of base Y adjacent to the anticodon of yeast phenylalanine tRNA changes the coding properties of tRNA\textsuperscript{Phe}. Phenylalanine tRNA from which base Y has been removed (tRNA\textsuperscript{Y}) recognizes UUC better than UUU. Codon UUU is read efficiently at higher Mg\textsuperscript{2+} concentration or in the presence of streptomycin. A dipeptide cannot be synthesized with N-acetyl-Phe-tRNA\textsuperscript{Phe} and Phe-tRNA\textsuperscript{Phe} unless the base Y is also removed from the acetylated Phe-tRNA. Removal of base Y from the anticodon loop does not affect the ability of phenylalanyl-tRNA\textsuperscript{Phe} to form the ternary complex with GTP and the bacterial elongation factor T.

Modified nucleosides have been found in tRNA isolated from bacteria and higher organisms (1, 2). In the tRNA molecule these modified components are located mainly in the nonhydrogen-bonded areas. A modified base occurs adjacent to the 3' end of the anticodon of most tRNA's and there seems to be a structural correlation between the 3' end base of the anticodon and the adjacent modified base. For example, Escherichia coli tRNA anticodons that have adenosine as the 3' end base contain the modified adenosine derivative, 2-thiomethyl-N\textsuperscript{6}-(\Delta\textsuperscript{2}-isopentenyl)adenosine (1, 2). In yeast and rat liver tRNA, N\textsuperscript{6}-(\Delta\textsuperscript{2}-isopentenyl)adenosine occurs in the corresponding positions (1, 2). An exception is yeast and wheat germ tRNA\textsuperscript{Phe} which contains the base Y in this position (3-5). Similarly with tRNA species that have uridine as the 3' end base of the anticodon, N-(purin-6-yl-carbamoyl)threonine occurs in the corresponding position (1, 6).

The function of the modified nucleoside next to the anticodon has been studied. Selective iodination of the isopentenyl group of the N\textsuperscript{6}-(\Delta\textsuperscript{2}-isopentenyl)adenosine residue in Ser-tRNA\textsuperscript{Ser} reduced the capacity of this tRNA to bind to a poly(U)-ribosome complex (7). In similar studies, base Y was removed from tRNA\textsuperscript{Phe} by mild acid treatment (8) and this treatment led to loss of capacity to bind to a poly(U)-ribosome complex and to transfer phenylalanine to a polypeptide but had no effect on the aminocacylation of the tRNA. In other studies, it was shown that both the 2-thiomethyl and \Delta\textsuperscript{2}-isopentenyl groups of the modified adenosine adjacent to the anticodon of tRNA\textsuperscript{Phe} were necessary for optimum binding of the tRNA molecule to the mRNA-ribosome complex (9). The presence of a specially modified nucleoside adjacent to the anticodon thus seems essential for the proper functioning of some tRNA species.

In this report we show that removal of base Y from tRNA\textsuperscript{Phe} of yeast and wheat germ changes its coding properties perhaps due to an altered conformation of the anticodon loop. Our results also show that the conformation of the anticodon loop or the presence of a modified base adjacent to the anticodon is not involved in the binding of aminocacyl-tRNA by transfer factor T.

Preliminary reports of these studies have been made (10, 11).

MATERIALS AND METHODS

Uniformly \textsuperscript{14}C-labeled L-amino acids, \textsuperscript{3}H-labeled amino acids, and nucleoside triphosphates were obtained commercially. Benzoylated DEAE-cellulose and benzoylated DEAE-cellulose on silicic acid were prepared as described by Gillam et al. (12) and Wimmer, Maxwell, and Tener (13). Fusidic acid was a gift from Dr. W. O. Godtfredsen of Leo Pharmaceutical Products, Denmark, and streptomycin was supplied by Merck Sharpe & Dohme. N-Acetyl phenylalanine, phenylalanylphenylalanine, and phenylalanylphenylalanylphenylalanine were from Sigma. N-acetyl-Phe-Phe and N-acetyl-Phe-Phe-Phe were prepared from Phe-Phe and Phe-Phe-Phe as described by Greenstein and Winitz (14).

Counting and Scanning of Radioactivity—Paper discs and paper chromatograms or electropherograms after cutting into strips, 1 \times 2.5 cm, were counted in a scintillation fluid containing 4 g of 2,5-diphenyloxazole (POPOP) per liter of toluene. The counting efficiency for \textsuperscript{3}H was 5% and that for \textsuperscript{14}C was 65%. Millipore filters were counted in 10 ml of Bray's solution (15) after they were completely dissolved and stored in the dark for at least 6 hours. The counting efficiencies of \textsuperscript{3}H and \textsuperscript{14}C under these conditions were 25 and 80%, respectively.

Paper Chromatography—The ascending paper technique was used at room temperature with Whatman No. 1 filter paper. The solvent systems used were Solvent A, 1-butanol-glacial acetic acid-water (78:5:17) and Solvent B, 1-butanol saturated with 1 M ammonium hydroxide.
Silicic acid chromatography were performed at room temperature (12, 13). The fractions were assayed directly with a yeast synthetase preparation free of tRNA. Appropriate fractions were precipitated with 2 volumes of alcohol in the presence of 1 M NaCl. For tubes with an A260 of less than 5, pooled fractions were evaporated under reduced pressure to one-third of the original volume and the tRNA was then precipitated with alcohol.

**Poly nucleotides—**Poly(U) was obtained by the reaction of DNA-dependent RNA polymerase with poly[d(T-T-C)]-poly[d(G-A-A)] as template (17). DNA templates were replicated with DNA polymerase (18).

**Enzymes—**Pancreatic DNase, electrophoretically pure and free of RNase was obtained from Worthington; rabbit muscle pyruvate kinase was obtained either from Sigma or from Calbiochem. DNA polymerase was prepared from E. coli (19) and was purified up to the phosphocellulose step. RNA polymerase was purified as described previously (17).

Crude yeast aminocyl synthetase was prepared according to the method of Hoskinson and Khorana (20). A partially purified yeast aminocyl synthetase free from tRNA was prepared according to the method of Nishimura et al. (21).

Crude wheat germ synthetase was prepared by blending commercial wheat germ with 0.02 M Tris-HCl (pH 7.5)-0.01 M MgCl2-0.05 M KCl-0.005 M 2-mercaptoethanol and dialyzed against the same buffer for 4 hours. The dialyzed fraction was made up to 50% glycerol by adding cold glycerol and stored at -20°C. The resulting supernatant fraction was fractionated with solid (NH4)2SO4 and the fraction precipitating between 40 to 70% saturation of (NH4)2SO4 was collected. The precipitate was dissolved in a buffer containing 0.01 M Tris-HCl (pH 7.5)-0.005 M MgCl2-0.05 M KCl-0.01 M 2-mercaptoethanol and dialyzed against the same buffer for 4 hours. The dialyzed fraction was made up to 50% glycerol by adding cold glycerol and stored at -20°C.

**tRNA and Aminoacyl-tRNA—**tRNA was isolated from bakers’ yeast according to the procedure of Holley (22). The crude yeast tRNA was fractionated on a BD-cellulose column (12). The fraction eluted by 15% alcohol was precipitated with 2 volumes of alcohol, centrifuged, and used for further purification of tRNAphe. The partially purified yeast tRNAphe was further purified on a BD-cellulose column with a linear gradient of 0.01 M sodium acetate (pH 5.0)-0.8 M NaCl in the mixing chamber and 0.01 M sodium acetate (pH 5.0)-1.5 M NaCl containing 15% ethanol in the reservoir. The purified tRNAphe could accept 1.1 to 1.4 nmoles of phenylalanine per A260 unit. Wheat germ tRNAphe was prepared with the same procedure as described for yeast tRNAphe (8). The preparations of tRNAphe were free from tRNAphe as shown by the fluorescence spectra (23).

**Preparation of Phenylalanyl-tRNA—**Phenylalanyl-tRNA was synthesized and isolated as described earlier (17) with the modification that the reaction mixture contained 0.05 M sodium cacodylate (pH 7.4), 2 mM ATP, and 20 mM MgCl2. Wheat germ phenylalanyl-tRNA was prepared with wheat germ synthetase. The procedure described for yeast Phe-tRNA preparation was followed except that the reaction mixture contained 0.1 M Tris-HCl (pH 7.1), 5 mM MgCl2, and 5 mM ATP.

**Acetylation of Phenylalanyl-tRNA—**Phenylalanyl-tRNA samples were acetylated with acetic anhydride at 4°C (24). The acetylated products were examined chromatographically or electrophoretically after alkaline hydrolysis and were shown to be free of phenylalanyl-tRNA.

**Ribosomes and Supernatant Factors—**Ribosomes were isolated from E. coli MRE 600 and washed with NH4Cl (25). The supernatant fractions (6-100) were freed from tRNA by passage through a DEAE-cellulose column (27). The amino acid polymerizing factors T and G were purified from the supernatant fraction by separation on a DEAE-sephadex column (25) after removal of nucleic acids using a phase partition system (26).

A ribosomal system for protein synthesis from wheat germ was obtained by modification of the procedure described by Allende and Bravo (28). Ribosomes were isolated from commercial wheat germ and washed twice with the extracting medium (0.5 M sucrose 0.01 M Tris-HCl (pH 7.5)-0.01 M magnesium acetate-0.05 M KCl-0.005 M 2-mercaptoethanol containing 0.2 M KCl. The clear S-100 fraction from wheat embryo extract was fractionated with solid (NH4)2SO4 and the fraction precipitating between 40 to 70% saturation of (NH4)2SO4 was collected. The precipitate was dissolved in a buffer containing 0.02 M Tris-HCl (pH 7.5)-0.005 M magnesium acetate-0.05 M KCl-0.01 M 2-mercaptoethanol and dialyzed against the same buffer for 4 hours.

**Assay for Amino Acid Acceptor Activity—**The reaction mixture was identical with that described for the preparation of amino-acyt-la-tRNA except that the final volume was 0.1 ml and, after 10 min at 37°C, a 0.05-mL aliquot was withdrawn. Acid-insoluble radioactivity was measured on filter paper as described previously (17).

**Assay for in Vitro Amino Acid Incorporation—**As in the previous work (17) a two-step procedure was used. Stage I involved the synthesis of a polyribonucleotide with DNA-like polymers as template for RNA polymerase; in Stage II the polyribonucleotide was used without isolation for polypeptide synthesis.

**Assay of Complex Formation between T, GTP, and Aminoacyl-tRNA—**The complex formed between elongation factor T, GTP, and phenylalanyl-tRNA was assayed (a) by gel filtration (29) and (b) by Millipore filtration (30). In the Millipore filter technique [3H]GTP was previously incubated with a triphosphate-regenerating system to convert any contaminating [3H]GDP into [3H]GTP (30).
Assay of Aminoacyl-tRNA Binding to Ribosomes and Synthesis of Dipeptide—The general procedure of Nirenberg and Leder (31) was followed. Specific binding of aminoacyl-tRNA to the ribosome-mRNA complex containing peptidyl-tRNA was measured by the two-step-binding procedure (16). The dipeptide synthesized in the two-step-binding reaction was followed by using Complex I containing N-Ac-[αH]phenylalanyl-tRNA and Complex II containing [14C]Phe-tRNA, and the nature of the labeled compound bound to the ribosome was determined (16). Peptidyl-tRNA was assayed by the puromycin reaction (16).

RESULTS

Coding Properties of tRNA^{Phe}_Y—The effect of removal of the base Y on the specificity of codon recognition of tRNA^{Phe} was studied by comparing the transfer of phenylalanine from [14C]Phe-tRNA^{Phe} and [14C]Phe-tRNA^{Phe}_Y into polyphenylalanine with both poly(U) and poly[ur(U-U-C)] as messenger in E. coli cell-free extracts. Fig. 1 shows that poly(U-U-C) is translated by both Phe-tRNA^{Phe} and Phe-tRNA^{Phe}_Y, but poly(U) is read only by Phe-tRNA^{Phe} at 10 mM Mg^{++} concentration. The effect of different Mg^{++} concentrations on the polymerization of phenylalanine directed by both the templates is shown in Fig. 2. [14C]Phe-tRNA^{Phe}_Y transfers phenylalanine into polypeptide directed by poly(U) at higher Mg^{++} concentrations. These results show that tRNA^{Phe}_Y can recognize UUC at a lower Mg^{++} concentration than it recognizes UUU but both UUU and UUC are read efficiently by tRNA^{Phe} at lower Mg^{++} concentrations.

Effect of Streptomycin on Coding Properties of tRNA^{Phe}_Y—Translation of the codon UUU at a higher Mg^{++} concentration might be due to the misreading caused by high Mg^{++} concentrations (32). Since streptomycin is also known to induce mis-coding (33), the effect of streptomycin on phenylalanine transfer at different Mg^{++} concentrations was examined. The results are shown in Table I. The presence of streptomycin inhibited the incorporation of phenylalanine from Phe-tRNA^{Phe}_Y in presence of both poly(U) and poly[ur(U-U-C)]. Incorporation of phenylalanine from Phe-tRNA^{Phe}_Y with poly(U) as template was significantly increased by the presence of streptomycin. The preferential reading of the UUC codon by tRNA^{Phe}_Y under normal conditions of low Mg^{++} concentrations could be due to an altered conformation of the anticodon (2'-OMeGAA) of tRNA^{Phe}_Y which does not permit the nonstandard base pairing (34) between...

![Fig. 1. Phenylalanine polymerization from yeast [14C]Phe-tRNA^{Phe} and [14C]Phe-tRNA^{Phe}_Y as directed by poly(U) and poly[ur(U-U-C)]. The reaction mixture in a volume of 1 ml contained 1.0 A260 unit of poly(U) or 5.4 nmoles of poly[ur(U-U-C)], 20 A260 units of washed ribosomes, and 0.4 mg of proteins containing T and G factors and was incubated at 37° for 120 min. Specific activity of [14C]phenylalanine used was 475 mCi per mmole.](http://www.jbc.org/)

![Fig. 2. Effect of Mg^{++} concentration on [14C]phenylalanine polymerization from [14C]Phe-tRNA^{Phe} and [14C]Phe-tRNA^{Phe}_Y as stimulated by poly(U) and poly[ur(U-U-C)]. Experimental conditions were the same as in Fig. 1 except that varying amounts of Mg^{++} ion and 350 pmoles of [14C]Phe-tRNA^{Phe} or 362 pmoles of [14C]Phe-tRNA^{Phe}_Y were present and time of incubation was 60 min.](http://www.jbc.org/)
Effect of streptomycin on phenylalanine polymerization at varying Mg++ concentration

Experimental conditions are described under Fig. 1 except that the Mg++ concentration was varied and streptomycin was added to 10 μg per ml.

### Table I

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Effect of Mg++ concentration and presence of streptomycin on phenylalanine polymerization from [3C]Phe-tRNA Phe and [3C]Phe-tRNA Phe using ribosomes from streptomycin-resistant strain of E. coli

The experimental conditions were the same as described in Fig. 1, except that 27 ASO units of ribosomes isolated from streptomycin-resistant mutant of E. coli (strain PR7) was present in 1 ml of reaction mixture which contained 615 pmoles of yeast tRNA Phe or 270 pmoles of yeast [3C]Phe-tRNA Phe and 20 μg of streptomycin as indicated. Time of incubation was 30 min.

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<tr>
<th>Mg++ concentration</th>
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<td>[3C]Phe-tRNA Phe</td>
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<td>poly(U)</td>
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<td>6.5</td>
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Increased binding capacity to ribosome-poly(U) complex even at higher Mg++ concentrations. Streptomycin had no effect on binding in either case. The increased binding of Phe-tRNA Phe at 20 or 30 mM Mg++ concentration could be due to a change in the conformation of tRNA Phe allowing the unfavorable G=U base pairing. The nonstandard base pairing between guanylate and uridylylate could also be stabilized by the increase in concentration of Mg++ ion.

**Table II**

Effect of Mg++ concentration and presence of streptomycin on poly(U)-directed phenylalanine polymerization from [3C]Phe-tRNA Phe and [3C]Phe-tRNA Phe using ribosomes from streptomycin-resistant strain of E. coli

The experimental conditions were the same as described in Fig. 1, except that 27 ASO units of ribosomes isolated from streptomycin-resistant mutant of E. coli (strain PR7) was present in 1 ml of reaction mixture which contained 615 pmoles of yeast [3C]Phe-tRNA Phe or 270 pmoles of yeast [3C]Phe-tRNA Phe and 20 μg of streptomycin as indicated. Time of incubation was 30 min.

<table>
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<th>Mg++ concentration</th>
<th>[3C]Phenylalanine polymerized</th>
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<td>[3C]Phe-tRNA Phe</td>
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<td>poly(U)</td>
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<tr>
<td>mM</td>
<td>pmole/ml</td>
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Increased binding capacity to ribosome-poly(U) complex even at higher Mg++ concentrations. Streptomycin had no effect on binding in either case. The increased binding of Phe-tRNA Phe at 20 or 30 mM Mg++ concentration could be due to a change in the conformation of tRNA Phe allowing the unfavorable G=U base pairing. The nonstandard base pairing between guanylate and uridylylate could also be stabilized by the increase in concentration of Mg++ ion.

**Table III**

Effect of streptomycin on binding of phenylalanyl-tRNA to ribosomes in the presence of poly(U) at different Mg++ concentration

The reaction mixture contained in 1 ml, 180 pmoles of [3C]Phe-tRNA Phe or 190 pmoles of [3C]Phe-tRNA Phe and 10 μg of streptomycin as indicated.

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<td>poly(U)</td>
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<td>pmole/ml</td>
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<td>5</td>
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<td>20</td>
<td>6.3</td>
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<td>10.7</td>
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**Table IV**

Binding of Phe-tRNA Phe to ribosome-poly(U) complex containing N-acetyl-Phe-tRNA and synthesis of dipeptide

The two-stage assay procedure is described under "Materials and Methods." In Stage I, ribosome-poly(U) complex containing N-acetyl phenylalanyl-tRNA (Complex I) and the T-GTP-Phe tRNA complex (Complex II) were synthesized separately. Reaction mixtures for Complex I formation contained per ml, 31 A260 units of ribosomes, 4 A260 units of poly(U), 256 pmoles of Ac-[3H]Phe-tRNA Phe or 270 pmoles of Ac-[3H]Phe-tRNA Phe and magnesium acetate as indicated. The reaction mixture for Complex II synthesis contained in 1 ml, 435 pmoles of [3C]Phe-tRNA Phe and other components described under "Materials and Methods." In Stage I, equal volumes of Complex I and II were mixed. Specific activities of [3H]phenylalanine and [3C]phenylalanine were 2100 and 475 mCi per mmole, respectively. The procedures for the determinations of aminoacyl-tRNA bound to ribosomes, acetyl phenylalanylpuromycin formed, and the dipeptide synthesized are described under "Materials and Methods."
to Ribosome—An altered conformation of the anticodon loop of tRNA\textsubscript{Phe} might create a steric hindrance between itself and the anticodon loop of tRNA\textsubscript{Phe} when they bind to the same ribosome. We, therefore, tested the enzymatic binding of Phe-tRNA\textsubscript{Phe} to the ribosome-poly(U) complex containing N-Ac-Phe-tRNA\textsubscript{Phe} or N-Ac-Phe-tRNA\textsubscript{Phe}. At 10 mM Mg\textsuperscript{2+} concentration elongation factor, T, is required to bind Phe-tRNA\textsubscript{Phe} to the ribosomepoly(U) complex containing N-Ac-Phe-tRNA\textsubscript{Phe}. Phenylalanyl-tRNA\textsubscript{Phe} could, however, bind to the ribosome-poly(U) complex containing N-Ac-Phe-tRNA\textsubscript{Phe}. The presence of both N-Ac-Phe-tRNA\textsubscript{Phe} and Phe-tRNA\textsubscript{Phe} on a ribosome results in the synthesis of a dipeptidyl-tRNA (N-Ac-Phe-Phe-tRNA\textsubscript{Phe}) (16). The results presented in Table IV show that in presence of N-Ac-Phe-tRNA\textsubscript{Phe} very little binding of Phe-tRNA\textsubscript{Phe} occurred and no dipeptide N-Ac-Phe-Phe was formed. Increasing the Mg\textsuperscript{2+} concentration failed to stimulate the synthesis of dipeptide. The dipeptide N-Ac-Phe-Phe was synthesized only when Phe-tRNA\textsubscript{Phe} was bound to the ribosome-poly(U) complex containing N-Ac-Phe-tRNA\textsubscript{Phe}. These data suggest that both tRNA\textsubscript{Phe} and tRNA\textsubscript{Phe} can not bind to the same ribosome to form a dipeptide, possibly due to a steric hindrance occurring between the anticodon loops of the 2 tRNA molecules on the ribosome.

Recognition of tRNA\textsubscript{Phe} by Elongation Factor T—Enzymatic binding of aminoacyl-tRNA to the ribosome-poly(U) complex involves the formation of the complex, T-GTP-aminoacyl-tRNA. The observed decreased binding of Phe-tRNA\textsubscript{Phe} might be due to decreased formation of the T-GTP-Phe-tRNA\textsubscript{Phe} complex. Fig. 3 shows that in presence of T and [\textsuperscript{3}H]GTP both Phe-tRNA\textsubscript{Phe} and Phe-tRNA\textsubscript{Phe} form the ternary complex as determined by Sephadex G-100 chromatography. No difference

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**Fig. 3.** Complex formation between T, GTP, and phenylalanyl-tRNA as assayed by Sephadex gel filtration. In A, the reaction mixture contained, in addition to other components, 5 nmoles of [\textsuperscript{3}H]GTP (specific activity, 1000 mCi per mmole) and 232 pmoles of [\textsuperscript{14}C]phenylalanyl-tRNA\textsubscript{Phe} in absence of T factor. B and C contained, in addition to 5 nmoles of [\textsuperscript{3}H]GTP and 200 \mu g of T factor, 137 pmoles of [\textsuperscript{14}C]Phe-tRNA\textsubscript{Phe} and 232 pmoles of [\textsuperscript{14}C]Phe-tRNA\textsubscript{Phe}, respectively.
was present at a concentration of 20 pg per ml.

The reaction mixture in a volume of 1 ml contained 310 pmoles of wheat germ [14C]Phe-tRNAPhe or 360 pmoles of wheat germ [14C]Phe-tRNA\textsuperscript{hy}e and was incubated for 60 min. Streptomycin was present at a concentration of 20 μg per ml.

| Mg\textsuperscript{++} concentration | Ribosome used | [14C]Phenylalanine polymerized | [3H]GTP, and either Phe-tRNAPhe or Phe-tRNA\textsuperscript{hy}e was found. Control experiments showed that active in polyphenylalanine synthesis directed by poly(U) and tRNA\textsuperscript{hy}e was used. Unlike the E. coli ribosomal system the presence of streptomycin had no effect. However, an in-stable base pair with uridylicate. Therefore, UUC is recognized by tRNA\textsuperscript{hy}e only. In fact, the codon UUC has been shown to bind to the tRNA\textsuperscript{hy}e anticodon 5 times as strongly as the codon UUU (39). Increasing the concentration of Mg\textsuperscript{+++} ion may stabilize G=U base pairing by a change in the conformation of tRNA and thus UUU can be recognized by tRNA\textsuperscript{hy}e. Ohashi et al. (40) have shown that the anticodon of E. coli tRNA\textsuperscript{hy}e contains a 2-thiouridine derivative in the wobble position. At low Mg\textsuperscript{+++} concentration only GAA is recognized but on increasing the concentration of Mg\textsuperscript{+++} ion GAG is also recognized. The recognition of codons containing a wobble base at the third position by tRNA at increased Mg\textsuperscript{+++} concentrations may be due to changes in the conformation of a tRNA molecule induced by Mg\textsuperscript{+++} ions (41). With the fluorescence of Y as a marker it was shown that the conformation of the anticodon loop of tRNAPhe 1s dependent on the presence of Mg\textsuperscript{+++}

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<th>Ribosome used</th>
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<td>2.6 5.3 7.3</td>
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<tr>
<td>10 E. coli</td>
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<td>20 E. coli</td>
<td>10.9 27.8</td>
<td>11.4 128.4 195</td>
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in the kinetics of formation of the ternary complex between T, [PH]GTP, and either Phe-tRNAPhe or Phe-tRNA\textsuperscript{hy}e was found. The results would thus suggest that the base adjacent to the 3’ end of the anticodon as well as the conformation of the anticodon may not be recognized by T factor. A recent report (38) also shows that integrity of the anticodon loop is not necessary for T factor recognition. Chen and O’fargand (37), however, observed that the 2’,3’-carbon-carbon bond of the ribose of the 3’-terminal adenylate residue of the tRNA molecule is required for T-GTP-aminocyl-tRNA complex formation.

Role of Ribosomes in Codon-Anticodon Recognition—Codon-anticodon interaction is determined mainly by the base-pairing principles but the ribosome can also participate in control of this process (35). In an effort to study the role of the ribosome we tested polyphenylalanine synthesis in the 80 S ribosomal system from wheat germ with uncharged phenylalanine tRNA. At 10 mM Mg\textsuperscript{++} wheat germ tRNAPhe is very active in polyphenylalanine synthesis directed by poly(U) and the presence of streptomycin had no effect. However, an insignificant amount of polyphenylalanine was formed when wheat germ tRNA\textsuperscript{hy}e was used. Unlike the E. coli ribosomal system addition of streptomycin or increasing the Mg\textsuperscript{++} concentration showed no stimulatory effect. Control experiments showed that wheat germ tRNA\textsuperscript{hy}e could be fully charged under these conditions. The failure of wheat germ tRNA\textsuperscript{hy}e to recognize UUU could thus be an intrinsic property of wheat germ tRNA\textsuperscript{hy}e itself. Wheat germ ribosomes could also restrict the recognition of UUU by the modified anticodon conformation of wheat germ tRNA\textsuperscript{hy}e. To test these two possibilities homologously charged wheat germ Phe-tRNAPhe or Phe-tRNA\textsuperscript{hy}e was used for poly(U)-directed polyphenylalanine synthesis with both wheat germ ribosomes and E. coli ribosomes. Results presented in Table V show that in an E. coli ribosomal system wheat germ Phe-tRNAPhe could recognize UUU in the presence of streptomycin or at a higher Mg\textsuperscript{++} concentration.

DISCUSSION

In an effort to understand the role of the modified base adjacent to the anticodon we have removed the modified base Y from yeast or wheat germ tRNA\textsuperscript{hy}e and studied the coding properties of tRNA\textsuperscript{hy}e. Other workers reported that this modified base is involved in the binding of aminoacyl-tRNA to the mRNA-ribosome complex and in the ability of the tRNA to transfer amino acid into poly peptides (7–9, 36). Our amino acid incorporation studies further show that removal of Y affects the codon-anticodon interaction of tRNAPhe. Thus, at a low Mg\textsuperscript{++} concentration (10 mM) tRNA\textsuperscript{hy}e recognizes UUC but not UUU. Only at higher Mg\textsuperscript{++} concentration are both the codons recognized. The modified base adjacent to the anticodon, therefore, may play a significant role in the codon-anticodon recognition process.

It is generally believed that codon recognition at lower Mg\textsuperscript{++} concentration, rather than at high Mg\textsuperscript{++}, may represent the true in vivo situation. Thus, removal of Y may affect the conformation of tRNA\textsuperscript{hy}e such that the 5’ base of the anticodon (2’-OMeG) can not form a stable base pair with uridylicate. Therefore, UUC is recognized by tRNA\textsuperscript{hy}e only. In fact, the codon UUC has been shown to bind to the tRNA\textsuperscript{hy}e anticodon 5 times as strongly as the codon UUU (39). Increasing the concentration of Mg\textsuperscript{+++} ion may stabilize G=U base pairing by a change in the conformation of tRNA and thus UUU can be recognized by tRNA\textsuperscript{hy}e. Ohashi et al. (40) have shown that the anticodon of E. coli tRNA\textsuperscript{hy}e contains a 2-thiouridine derivative in the wobble position. At low Mg\textsuperscript{+++} concentration only GAA is recognized but on increasing the concentration of Mg\textsuperscript{+++} ions GAG is also recognized. The recognition of codons containing a wobble base at the third position by tRNA at increased Mg\textsuperscript{+++} concentrations may be due to changes in the conformation of a tRNA molecule induced by Mg\textsuperscript{+++} ions (41). With the fluorescence of Y as a marker it was shown that the conformation of the anticodon loop of tRNAPhe is dependent on the presence of Mg\textsuperscript{+++} ions (23). Recognition of the weak codon in the presence of high Mg\textsuperscript{+++} ion could also be due to misreading induced by a change in ribosomal conformation. Increasing the Mg\textsuperscript{+++} concentrations in a ribosomal system is known to increase translational ambiguity (32).

Recognition of UUU by tRNA\textsuperscript{hy}e at low Mg\textsuperscript{++} concentration is enhanced by the presence of streptomycin. It is now believed that streptomycin induces conformational changes in ribosomes which allow ambiguous translation (33, 42). The recognition of UUU by tRNA\textsuperscript{hy}e in the presence of streptomycin could thus be made possible by misreading the codon UUU as UUC. This possibility could be tested by using ribosomes from streptomycin-resistant strains of E. coli. The studies of Gorini (35), and Biswas and Gorini (43) with ribosomal mutants showed that ribosomes significantly influence codon-anticodon interactions. Thus, strA ribosomal mutants restrict mistranslation and raw ribosomal mutants reverse the restrictions imposed by the strA mutation and allow increased levels of ambiguous translation. As shown in Table II, addition of streptomycin to ribosomes from strA mutants did not stimulate the recognition of UUU by tRNA\textsuperscript{hy}e. Ribosomes from strA mutants, however, allowed tRNA\textsuperscript{hy}e to recognize UUU at higher Mg\textsuperscript{+++} concentration. Studies with ribosomes isolated from raw mutants showed that higher Mg\textsuperscript{+++} concentration or presence of streptomycin at lower Mg\textsuperscript{+++} concentration is required for poly(U)-directed phenylalanine incorporation from tRNA\textsuperscript{hy}e.2 These results suggest that the increased level of mistranslation by the ribosome in presence of streptomycin can not alone explain the recognition of UUU by tRNA\textsuperscript{hy}e. The presence of streptomycin or in-

\[2\textsuperscript{nd} Unpublished observations.\]
increased Mg$^{2+}$ concentration possibly permits or stabilizes the weak codon-anticodon interaction occurring between the disfavored codon UUU and the altered conformation of the anticodon loop of tRNA$^{Ph}$.

In the model of the anticodon loop proposed by Fuller and Hodgson (44) the modified base adjacent to the 3' end of the anticodon is part of a regular stacked series of nucleotides with the 5' base (wobble base) of the anticodon at the top. Support for this type of conformation has been obtained by fluorescence measurements on tRNA$^{Ph}$ (23). Using model synthetic oligonucleotides, Leonard et al. (45) showed that the modified base increases the stabilization of the stacked conformation of the anticodon loop as suggested by Fuller and Hodgson (44). Removal of the base Y might flip the anticodon loop structure so that the pyrimidines adjacent to the 5' end of the anticodon are involved in stacking (see Fig. 2 in Reference 10). This alternate conformation would, however, allow less conformational flexibility as the 5' base in the Fuller-Hodgson model (44). The 3' base of the anticodon would now have the same degree of flexibility as the 5' base of the anticodon loop of tRNA$^{Ph}$, (23, 41). The inability of tRNA$^{Ph}$ and tRNA$^{Ph}$ to bind to the same ribosome and form a dipeptide also strongly suggest that tRNA$^{Ph}$ and tRNA$^{Ph}$ have different conformations. The presence of N as Phe of tRNA$^{Ph}$ may thus sterically block the binding of Phe-tRNA$^{Ph}$ with an alternate anticodon loop conformation from form in any dipeptide. Our results thus suggest that removal of the b$^{16}$se adjacent to the 3' end of anticodon changes the conformation of the anticodon loop to an alternate Fuller-Hodgson structure. An alternate Fuller-Hodgson model of anticodon loop conformation is also suggested by other workers (36, 46, 47).

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Role of Modified Nucleosides in Transfer Ribonucleic Acid: EFFECT OF REMOVAL OF THE MODIFIED BASE ADJACENT TO 3' END OF THE ANTICODON IN CODON-ANTICODON INTERACTION
Kakoli Ghosh and Hara Prasad Ghosh


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