Relationship between Peptidyl Transferase Activity and Interaction of Ribosomes with Phenylalanyl Transfer Ribonucleic Acid–Guanosine 5′-Triphosphate–TI_u Complex*

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ABSTRACT: Evidence is presented which indicates that the interaction of a ribosome–poly(U) complex carrying *N*-acetylphenylalanyl-tRNA at the donor site with a phenylalanyl-tRNA–GTP–TI_u complex (complex II) is affected by the peptidyl transferase activity of the ribosome. Ribosome–poly(U)–*N*-acetylphenylalanyl-tRNA complexes prepared and isolated in the presence of NH_4^+ and K^+ rapidly interact with puromycin at 0° to form *N*-acetylphenylalanylpuromycin. Interaction of these ribosomes with complex II results in the binding of phenylalanyl-tRNA to the ribosomes, the formation of *N*-acetylphenylalanylphenylalanyl-tRNA, and the hydrolysis of approximately one guanosine 5′-triphosphate for each phenylalanyl-tRNA bound. Removal of NH_4^+ and K^+ from the ribosomal complex inactivates the peptidyl transferase, *i.e.*, very little *N*-acetylphenylalanylpuromycin is formed at 30° without NH_4^+ or at 0° with NH_4^+. When ribosomal complexes depleted of NH_4^+ and K^+ interact with complex II at 30°, less phenylalanyl-tRNA is bound, very little *N*-acetylphenylalanylphenylalanyl-tRNA is formed, and the hydrolysis of guanosine 5′-triphosphate is greatly increased. In addition, the binding of phenylalanyl-tRNA to the ribosomes is unstable. Heating the ribosomal complex with NH_4^+ (or K^+) restores the peptidyl transferase activity of the ribosomes, enhances the binding of phenylalanyl-tRNA, and restores the ratio of guanosine 5′-triphosphate hydrolyzed to phenylalanyl-tRNA bound to approximately one. Treatment of the ribosomes with sparsomycin, an antibiotic that inhibits the peptidyl transferase activity of the 50S ribosomal subunit, also decreases the binding of phenylalanyl-tRNA to the ribosomes and increases the hydrolysis of guanosine 5′-triphosphate. These results indicate that inactivation of the peptidyl transferase, either by removal of NH_4^+ and K^+ or by treatment with sparsomycin, not only prevents peptide bond formation but also decreases the binding of aminoacyl-tRNA at the acceptor site and increases the hydrolysis of guanosine 5′-triphosphate.

In bacterial systems binding of aminoacyl-tRNA at the acceptor site of a ribosome–mRNA complex occurs at low concentrations of Mg^{2+} in the presence of GTP and transfer factors, TI_s and TI_u[1] or in the presence of a complex designated complex II that is composed of aminoacyl-tRNA, GTP, and TI_u (for a recent review, see Lengyel and Söll, 1969). When Phe-tRNA from complex II is bound to a ribosome–poly (U) complex carrying AcPhe-tRNA at the donor site, most of the Phe-tRNA bound to the ribosomes interacts with the AcPhe-tRNA to form AcPhe-Phe-tRNA at the acceptor site (Lucas-Lenard and Haenni, 1968; Haenni and Lucas-Lenard, 1968; Ono *et al.*, 1969a; Ravel *et al.*, 1970; Shorey *et al.*, 1970). Approximately one GTP is hydrolyzed for each Phe-tRNA bound to the ribosomes (Shorey *et al.*, 1969; Ono *et al.*, 1969b; Gordon, 1969; Ravel *et al.*, 1970; Shorey *et al.*, 1970; Skoultchi *et al.*, 1970; Lucas-Lenard *et al.*, 1970). Recently Miskin *et al.* (1968) showed that peptidyl transferase, the portion of the 50S ribosomal subunit catalyzing peptide-bond formation (Monro and Vazquez, 1967), is inactivated when the ribosomes are depleted of NH_4^+ and K^+ and is rapidly reactivated when the ribosomes are incubated with NH_4^+ or K^+ at temperatures above 20°. In an earlier investigation in this laboratory (Ravel *et al.*, 1968) NH_4^+, and to a lesser extent K^+, was found to stimulate the GTP-dependent binding of Phe-tRNA to ribosomes catalyzed by TI (a mixture of TI_s and TI_u). NH_4^+ was also found to stimulate the formation of a Phe-tRNA–GTP–TI_u complex (complex II) which is an intermediate in the enzymatic binding reaction.

In this investigation we have determined the effects of NH_4^+ and sparsomycin on the interaction of complex II with ribosome–poly(U) complexes carrying AcPhe-tRNA at the donor site. Ribosome–poly(U)–AcPhe-tRNA complexes which have been depleted of K^+ and NH_4^+ bind less of the Phe-tRNA moiety of complex II and hydrolyze more of the GTP moiety of complex II. Very little, if any, of the Phe-tRNA is incorporated into dipeptide, and the binding of the Phe-tRNA to the ribosomes appears to be unstable. Heating the ribosomes with NH_4^+ *i.e.*, conditions that restore the peptidyl transferase activity of the ribosomes, enhances the amount of Phe-tRNA from complex II bound to the ribosomes and decreases the amount of GTP hydrolyzed, restoring the ratio of GTP hydrolyzed to Phe-tRNA bound to approximately 1. Sparsomycin, an antibiotic which inhibits peptide-bond formation by inhibiting the peptidyl transferase activity of the 50S

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[1] In an effort to establish a uniform nomenclature for the transfer factors, the following abbreviations are used: TI_s, TI_u, and TII. These correspond to the factors previously designated FI_s, FI_u, and FII (Shorey *et al.*, 1969) and have enzymatic activities comparable to factors T_s, T_u, and G (Lucas-Lenard and Lipmann, 1966) and S_1, S_3, and S (Skoultchi *et al.*, 1968). Also see Siler and Moldave (1970). Other abbreviations used are: Phe-tRNA, phenylalanyl transfer ribonucleic acid; AcPhe-tRNA, *N*-acetylphenylalanyl-tRNA; AcPhe-Phe, *N*-acetylphenylalanylphenylalanine; complex II, Phe-tRNA–GTP–TI_u.

ribosomal subunit (Goldberg and Mitsugi, 1967a,b; Monro and Vazquez, 1967; Lucas-Lenard and Haenni, 1968), also lowers the amount of Phe-tRNA bound to ribosome–poly U–AcPhe-tRNA complexes which have not been depleted of NH_4^+ and increases the amount of GTP hydrolyzed. These data indicate a relationship between the peptidyl transferase activity of the ribosomes and the interaction of the ribosomes with complex II which results in the binding of the aminoacyl-tRNA moiety and the hydrolysis of the GTP moiety.

Materials and Methods

Materials. Puromycin and chlortetracycline were purchased from Nutritional Biochemicals. Sparsomycin was kindly supplied by Dr. A. R. Stanley, Cancer Chemotherapy National Service Center, Bethesda, Md. Deoxycholate washed ribosomes and transfer factors, TI_s and TI_u, from *Escherichia coli* W were prepared as described previously (Ravel *et al.*, 1969; Ravel and Shorey, 1970). Initiation factors, f_1 and f_2, were isolated from the ammonium chloride wash of *E. coli* W ribosomes by the procedure of Erbe *et al.* (1969). [^{3}H]- and [^{14}C]Phe-tRNA and Ac[^{14}C]Phe-tRNA were prepared as previously described (Ravel *et al.*, 1967; Haenni and Chapeville, 1966).

Preparation of Ribosome–Poly(U)–AcPhe-tRNA Complexes. The reaction mixture contained buffer A-5 (Tris-HCl, pH 7.7, 0.05 M; dithiothreitol, 5 mM; NH_4Cl, 80 mM; KCl, 80 mM; and $MgCl_2$, 5 mM); ribosomes, 10 mg; poly(U), 1 mg; f_1, 300 μg of protein; f_2, 450 μg of protein; GTP, 70 nmoles; and Ac[^{14}C]Phe-tRNA, 3.5 nmoles, in a total volume of 7 ml. The mixture was incubated at 25° for 15 min and then cooled in an ice bath. One half of the mixture (3.5 ml) was layered over 6 ml of buffer A-5 containing 10% sucrose (ribosomes A); the other half was layered over 6 ml of buffer C-5 (Tris-HCl, pH 7.7, 0.05 M; dithiothreitol, 5 mM; and $MgCl_2$, 5 mM) containing 10% sucrose (ribosomes I). The ribosomes were collected by centrifugation at 150,000*g* for 90 min, and were stored as pellets at −90°. Just prior to use, ribosomes A were suspended in 0.4–0.8 ml of buffer B-5 (Tris-HCl, pH 7.7, 0.05 M; dithiothreitol, 5 mM; NH_4Cl, 160 mM; and $MgCl_2$, 5 mM) and ribosomes I were suspended in buffer C-5. Ribosomes R were obtained by heating ribosomes I in buffer B-5 for 5 min at 30°. Ribosome concentration was estimated from absorbance measurements at 260 mμ assuming that 14.4 A_{260} units equal 1 mg of ribosomes/ml. To determine the amount of Ac[^{14}C]-Phe-tRNA bound to the ribosomes, an aliquot containing 0.1–0.2 mg of ribosome was removed and diluted with buffer A-5 or C-5; the ribosomes were collected on a Millipore filter, washed with the appropriate buffer, and the amount of radioactivity retained by the Millipore filter was measured in a liquid scintillation counter.

Assays. Formation of AcPhe-puromycin. The reaction mixture contained 0.2 mg of the ribosome–poly(U)–Ac[^{14}C]-Phe-tRNA complex, 1 mM puromycin, and NH_4Cl, as indicated, in a total volume of 0.2 ml of buffer C-5. After incubation for 10 min at 0° or 5 min at 30°, 1.5 ml of ethyl acetate was added and the amount of AcPhe-puromycin present in the ethyl acetate phase was determined as described by Leder and Bursztyn (1966).

Binding of Phe-tRNA from complex II to ribosomes. Complex II containing TI_u, [^{3}H]Phe-tRNA, and [γ-^{32}P]GTP was prepared as described previously (Shorey *et al.*, 1969; Ravel and Shorey, 1970) except that the reaction mixture was incubated for 5 min at 25°. To remove K^+ and NH_4^+, the complex was dissolved in buffer C-5 and precipitated with ethanol before storage at −90°. The complex was dissolved in buffer C-5 just prior to use. The reaction mixture contained buffer C-5; NH_4Cl and complex II, as indicated; and 0.1 mg of the ribosome–poly(U)–Ac[^{14}C]Phe-tRNA complexes, in a final volume of 0.2 ml. The reaction mixture was incubated for 10 min at 0° or 5 min at 30°, and the amount of [^{3}H]Phe-tRNA from complex II bound to the ribosomes was determined by the Millipore filter method as described above.

Hydrolysis of the GTP moiety of complex II. The reaction mixture described for the binding assay was prepared in duplicate. One of the reaction mixtures was used to determine the amount of Phe-tRNA bound to the ribosomes and the other was used to determine the amount of [^{32}P]P_i liberated from the [γ-^{32}P]GTP by the procedure of Conway and Lipmann (1964).

Formation of AcPhe-Phe. The reaction mixture contained buffer C-5; 0.5 mg of ribosome–poly(U)–Ac[^{14}C]Phe-tRNA complexes, and complex II, as indicated, in a final volume of 0.5 ml. After 10-min incubation at 0° or 5 min at 30°, 0.1 mg of ribosomes was removed to determine the amount of [^{3}H]Phe-tRNA bound as described above. To the remainder of the reaction mixture 0.04 ml of 4 N KOH and a mixture of Phe, Ac-Phe, Phe-Phe, and Phe-Phe-Phe (20 μg each) were added. After heating at 50° for 15 min, 0.04 ml of 5 N acetic acid was added, and the hydrolysate was applied to a column of Sephadex G-15 (0.9 × 9 cm) equilibrated in 0.5 N acetic acid. The column was developed with 0.5 N acetic acid as described by Bretthauer and Golichowski (1964). The amount of AcPhe-Phe present in the hydrolysate was calculated from the amounts of Ac[^{14}C]Phe and [^{3}H]Phe eluted from the column between 8 and 12 ml.

Results

At low concentrations of Mg^{2+}, initiation factors 1 and 2, GTP and either K^+ or NH_4^+ are required for the poly(U)-directed binding of AcPhe-tRNA to *E. coli* ribosomes. In the following experiments AcPhe-tRNA was bound to the ribosome–poly(U) complexes in the presence of 5 mM $MgCl_2$, 80 mM KCl, and 80 mM NH_4Cl. Under these conditions approximately 10 pmoles of AcPhe-tRNA was bound per 0.1 mg of ribosomes and essentially all of the ribosome-bound AcPhe-tRNA interacted with puromycin at 0° to form AcPhe-puromycin. As shown in Table I when ribosomes prepared in this manner were harvested by centrifugation through a 10% solution of sucrose in buffer containing monovalent cations and were resuspended in buffer containing NH_4^+ (ribosomes A), they retained their ability to form AcPhe-puromycin at 0°. Maximal formation of AcPhe-tRNA at 0° was not obtained unless high concentrations of NH_4^+ (80 mM or higher) were also present in the incubation mixture. At 30° maximal formation of AcPhe-puromycin was obtained at lower concentrations of NH_4^+ (40–80 mM). When ribosomes carrying AcPhe-tRNA were harvested by centrifugation through a 10% solution of sucrose in buffer containing neither K^+ nor NH_4^+ and resuspended in buffer containing neither of the monovalent cations (ribosomes I), there was no detectable loss in the amount of AcPhe-tRNA bound to the ribosomes. These ribosomes were still capable of forming AcPhe-puromycin at

TABLE I: Formation of AcPhe-puromycin.[a]

Ribosome Prepn	NH_4^+ (mM)	pmoles of AcPhe-puromycin Formed/0.1 mg of Ribosomes 0°	30°
A	30[b]	4.0	8.7
A	160	10.7	11.0
I	0	<0.2	<0.2
I	160	0.6	8.0
R	27[b]	3.6	9.3
R	160	10.6	11.5

[a] Ribosomes A, I, and R carrying 9.7, 10.3, and 10.5 pmoles of Ac[^{14}C]Phe-tRNA per 0.1 mg, respectively, were prepared as described in Materials and Methods. [b] The final concentration of NH_4^+ in the standard reaction mixture was as indicated due to the presence of NH_4^+ in the ribosomal preparation.

TABLE II: Binding of Phe-tRNA Moiety of Complex II to Ribosomes and Formation of AcPhe-Phe.[a]

Ribosome Prepn	NH_4^+ (mM)	pmoles of Phe-tRNA/0.1 mg of Ribosomes Bound 0°	Incorpd into Dipeptide 0°	Bound 30°	Incorpd into Dipeptide 30°
A	30	6.3	2.3	10.4	4.1
A	160	7.3	3.4	11.6	4.5
I	0	5.3	<0.2	6.2	<0.2
I	160	7.4	0.7	12.3	5.3
R	27	5.4	2.0	9.2	4.5
R	160	6.4	3.5	10.8	4.9

[a] The standard reaction mixture was supplemented with 0.5 mg of the ribosomal complexes described in Table I and complex II containing 275 pmoles of [^{3}H]Phe-tRNA and 390 pmoles of [γ-^{32}P]GTP.

30° in the presence of NH_4^+ but were no longer capable of forming AcPhe-puromycin in 10 min at 0° even in the presence of high concentrations of NH_4^+. If ribosomes I were incubated with NH_4^+ for 5 min at 30° (ribosomes R), they regained their ability to transfer AcPhe to puromycin at 0° if high concentrations of NH_4^+ were also present during the interaction with puromycin. Complete restoration of peptidyl transferase activity was also obtained by incubating the ribosomes at 30° with K^+, but as reported earlier by Miskin *et al.* (1968), K^+ was less effective than NH_4^+. Although very little AcPhe-puromycin formation was observed with ribosomes I in 10 min at 0° in the presence of 160 mM NH_4^+, partial reactivation of peptidyl transferase (40–50%) was obtained when the ribosomes were incubated with 160 mM NH_4^+ at 0° for longer periods of time (40–60 min).

The effect of NH_4^+ on the binding of Phe-tRNA from complex II to ribosome–poly(U) complexes carrying AcPhe-tRNA at the donor site and on the formation of AcPhe-Phe is shown in Table II. In order to circumvent the effect of monovalent cations on the formation of complex II, complex II was prepared in the presence of NH_4^+ and K^+, and the monovalent cations were then removed by two precipitations of the complex with ethanol. Since the interaction of complex II with the ribosomal complex is so rapid (Gordon, 1969; Skoultchi *et al.*, 1970) and is essentially complete in less than 1 min at 30° or 0° either in the presence or absence of NH_4^+, the values reported in this investigation represent extents rather than rates of reaction. At 0° the amounts of Phe-tRNA from complex II bound to the three ribosomal preparations were not appreciably different and were stimulated only slightly by high concentrations of NH_4^+. As would be expected from the data given in Table I, there was a difference in the ability of the ribosomal preparations to form AcPhe-Phe. Both ribosomes A and ribosomes R formed AcPhe-Phe at 0°, although maximal formation was not obtained unless high concentrations of NH_4^+ were present during the interaction with complex II. With ribosomes I very little, if any, dipeptide was formed in the absence of NH_4^+, at either 0° or 30°. In the presence of NH_4^+ very little dipeptide was formed in 10 min at 0°; however, an increase in dipeptide formation was observed when the incubation was continued for longer periods of time. When no NH_4^+ was added, the amount of Phe-tRNA bound to ribosomes I at 30° was significantly lower than the amount bound in the presence of high concentrations of NH_4^+. The amount of Phe-tRNA bound to ribosomes I at 30° when no NH_4^+ was added varied considerably, ranging from as low as 4 pmoles to as high as 10 pmoles/0.1 mg of ribosomes. In all cases, however, the amount of Phe-tRNA incorporated into dipeptide was very small. Although the variation in the amount of Phe-tRNA bound to the ribosomes might be due to the presence of small amounts of monovalent cations, this variation is more likely due to the instability of the binding that occurs under these conditions, as will be discussed later. When ribosomes I were incubated at 30° in the presence of high concentrations of NH_4^+, there was an increase in the amount of Phe-tRNA bound to the ribosomes, and the amount of Phe-tRNA incorporated into dipeptide was about the same as that obtained with ribosome A or R.

The effect of NH_4^+ concentration on the binding of Phe-tRNA from complex II to ribosomes I and on the formation of AcPhe-Phe at 30° are shown in Figure 1. Increases in both binding and dipeptide formation were observed over the range of NH_4^+ concentrations tested, *i.e.*, 10–160 mM. The increase in the amount of Phe-tRNA bound to the ribosomes at the various concentrations of NH_4^+ paralleled the amount of Phe-tRNA incorporated into dipeptide. K^+ was found to be slightly more effective than NH_4^+ in stimulating the binding of Phe-tRNA to the ribosomes but less effective than NH_4^+ in promoting the formation of dipeptide.

In earlier investigations (Shorey *et al.*, 1969; Ravel *et al.*, 1970) it was shown that the GTP moiety of complex II was hydrolyzed when the aminoacyl-tRNA moiety of the complex was bound to the ribosomes and that the ratio of GTP hydrolyzed to aminoacyl-tRNA bound was approximately 1. All of these determinations were made in the presence of high

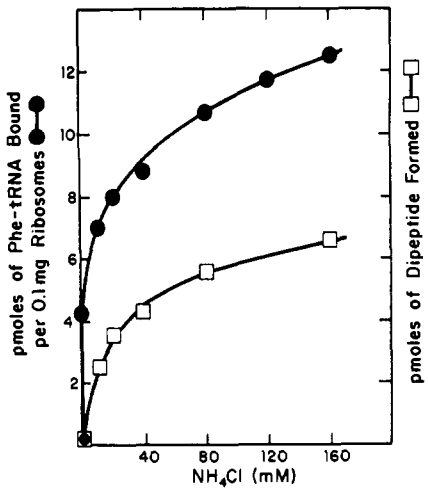

FIGURE 1: Effect of NH_4^+ concentration on the binding of Phe-tRNA from complex II to ribosomes and on the formation of AcPhe-Phe. The standard reaction mixture was supplemented with 0.5 mg of ribosomes I carrying 7.9 pmoles of Ac[^{14}C]Phe-tRNA/0.1 mg, complex II containing 210 pmoles of [^{3}H]Phe-tRNA and 330 pmoles of [γ-^{32}P]GTP, and NH_4Cl, as indicated, and was incubated at 30° for 5 min.

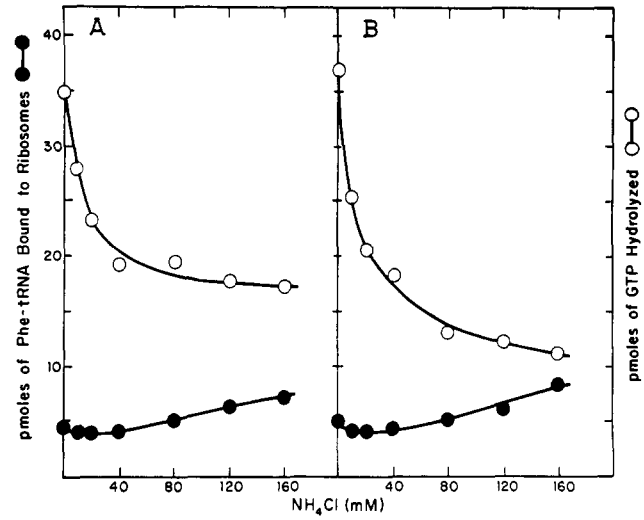

FIGURE 2: Effect of NH_4^+ concentration on the hydrolysis of the GTP moiety of complex II. The standard reaction mixture was supplemented with 0.1 mg of ribosomes I carrying 10.6 pmoles of Ac[^{14}C]Phe-tRNA, complex II containing 30 pmoles of [^{3}H]Phe-tRNA and 57 pmoles of [γ-^{32}P]GTP, and NH_4Cl, as indicated. In part A the reaction mixture was incubated for 2 min at 30°. In part B the reaction mixture, supplemented with NH_4Cl as indicated, was incubated for 5 min at 30° prior to the addition of complex II and then incubated for 2 min at 30°.

concentrations of NH_4^+. It was of interest, therefore, to determine the effect of NH_4^+ on the hydrolysis of the GTP moiety of complex II during the interaction of the complex with the ribosomes.

In the experiment described in Figure 2A, ribosomes depleted of NH_4^+ and K^+ (ribosomes I) were incubated at 30° for 2 min with complex II (containing [^{3}H]Phe-tRNA and [γ-^{32}P]GTP) at the specified NH_4^+ concentrations. When no NH_4^+ was added, the ratio of GTP hydrolyzed to Phe-tRNA bound to the ribosomes was greater than 8 to 1. Increasing the concentration of NH_4^+ increased the amount of Phe-tRNA bound to the ribosomes and decreased the amount of GTP hydrolyzed. At 160 mM NH_4^+, the ratio of GTP hydrolyzed to Phe-tRNA bound was reduced to approximately 2 to 1. In the experiment described in Figure 2B, ribosomes I were incubated for 5 min at 30° at the specified NH_4^+ concentrations prior to the addition of complex II; at 160 mM NH_4^+ the ratio of GTP hydrolyzed to Phe-tRNA bound approached 1. Incubation of the ribosomes with K^+ ion also decreased the amount of GTP hydrolyzed; however, K^+ was less effective than NH_4^+. In separate experiments with ribosomal preparations A or R, a minimum of 80 mM NH_4^+ was required in the incubation mixture to obtain levels of GTP hydrolysis comparable to the amount of Phe-tRNA bound.

To determine whether the effects observed with NH_4^+ were related to the peptidyl transferase activity of the ribosomes, a comparison of the effects of sparsomycin on the interaction of the ribosomes with puromycin and with complex II was made, and the results are shown in Figure 3. For these experiments the ribosome–poly(U) complexes carrying AcPhe-tRNA at the donor site were isolated in the presence of NH_4^+ (ribosomes A), and the reaction mixtures contained 160 mM NH_4^+. The data show that concentrations of sparsomycin which inhibited the formation of AcPhe-puromycin also decreased the binding of Phe-tRNA to the ribosomes and enhanced the hydrolysis of GTP. In separate experiments it was found that the formation of AcPhe-Phe was reduced from 4.5 to 1.4 pmoles per 0.1 mg of ribosomes in the presence of 1×10^{-7} M sparsomycin. These data indicate that inactivation of peptidyl transferase, either by removal of NH_4^+ and K^+ or by the addition of sparsomycin, decreases the amount of Phe-tRNA bound to the ribosomes and increases the amount of GTP hydrolyzed. A study of the effects of other antibiotics on the binding of aminoacyl-tRNA, dipeptide formation and GTP hydrolysis is in progress.

In a recent investigation (Shorey *et al.*, 1970) it was shown that almost none of the Phe-tRNA from complex II that was bound to ribosomes at the acceptor site prior to the addition of AcPhe-tRNA at the donor site was incorporated into dipeptide. It was therefore of interest to determine whether Phe-tRNA bound to ribosomes carrying AcPhe-tRNA at the donor site and having the peptidyl transferase inactivated by the removal of NH_4^+ would subsequently be incorporated into dipeptide upon the reactivation of the peptidyl transferase. When ribosomes I were incubated with complex II for 5 min at 30° in the absence of NH_4^+, and then incubated at 30° for an additional 5 min in the presence of NH_4^+, AcPhe-Phe was formed. Additional binding of Phe-tRNA occurred during the second incubation, however, and it was not possible to determine whether the Phe-tRNA incorporated into dipeptide arose from the Phe-tRNA bound to the ribosomes initially or from that bound during the second incubation. In order to separate the ribosomes from the complex II remaining after the first incubation, the ribosomes were isolated either by centrifugation through a 10% sucrose solution in buffer containing no NH_4^+ (buffer C-5) or by chromatography on Sephadex G-100 equilibrated in buffer C-5. During either procedure 60–70% of the Phe-tRNA and 40–50% of the

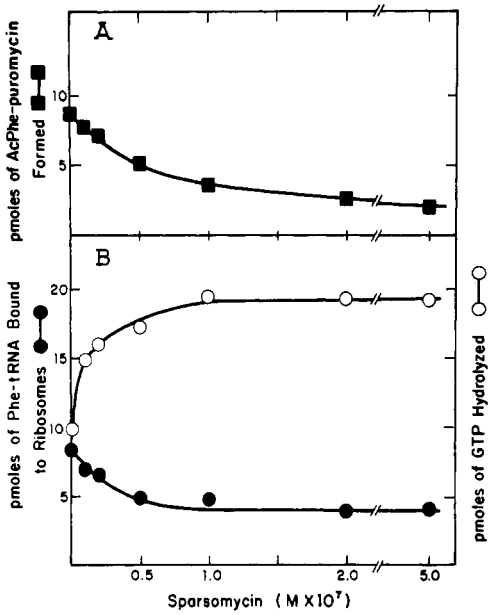

FIGURE 3: Effect of sparsomycin concentration on interaction with puromycin and complex II. The standard reaction mixture was supplemented with 0.1 mg of ribosomes A carrying 9.6 pmoles of Ac[^{14}C]Phe-tRNA, 160 mM NH_4Cl, and sparsomycin, as indicated, and was incubated for 2 min at 30°. In part A 1 mM puromycin was added and the reaction mixture was incubated for 10 min at 0°. In part B complex II containing 31 pmoles of [^{3}H]Phe-tRNA and 46 pmoles of [γ-^{32}P]GTP was added, and the reaction mixture was incubated for 2 min at 30°.

AcPhe-tRNA initially bound to the ribosomes (as measured by retention on a Millipore filter) was lost. The elution profile obtained by chromatography on Sephadex G-100 indicated that the Phe-tRNA was dissociating from the ribosomes during the gel filtration process. When the ribosomes, isolated by either procedure, were incubated with NH_4^+ at 30°, very little of the Phe-tRNA remaining bound to the ribosomes was incorporated into dipeptide, and most of the AcPhe-tRNA remaining bound to the ribosomes interacted with puromycin, indicating that the peptidyl transferase of the ribosomes had been reactivated. In other experiments, the Phe-tRNA from complex II was bound to ribosomes I in the absence of NH_4^+, chlortetracycline was added to prevent additional binding of Phe-tRNA, and the ribosomes were heated with NH_4^+. Very little dipeptide was formed under these conditions; however, a 60–70% loss in the amount of the Phe-tRNA bound to the ribosomes occurred in the presence of the chlortetracycline. Since so much of the Phe-tRNA dissociated from the ribosomes during the isolation procedures or in the presence of chlortetracycline, it is difficult to say whether or not dipeptide would have been formed had more of the Phe-tRNA remained bound to the ribosomes when the peptidyl transferase was reactivated. Futher investigation is necessary to resolve this question.

Discussion

The results of this investigation indicate that the interaction of complex II (Phe-tRNA–GTP–TI_u) with a ribosome–poly(U) complex carrying AcPhe-tRNA at the donor site is affected by the peptidyl transferase activity of the ribosomal complex. This conclusion is based upon two kinds of evidence, one the effect of NH_4^+ and, two, the effect of sparsomycin.

It was shown previously by Miskin *et al.* (1968) and Vogel *et al.* (1969) that the ability of the 50S ribosomal subunit to catalyze the formation of fMet-puromycin at 0° is lost if K^+ and NH_4^+ are removed from the ribosomes during the isolation procedure, and that this activity is rapidly restored if the ribosomes are heated with NH_4^+ or K^+. Similarly, Teraoka *et al.* (1970) showed that heating with high concentrations of K^+ or NH_4^+ restores the peptidyl transferase activity of ribosomes having an altered 50S ribosomal protein component. Ribosome–poly(U)–AcPhe-tRNA complexes isolated in the presence of K^+ and NH_4^+ (ribosomes A) interact with puromycin either at 0 or 30° to form AcPhe-puromycin. Interaction of complex II with these ribosomes results in the binding of Phe-tRNA to the ribosomes and in the formation of AcPhe-Phe-tRNA. The ratio of GTP hydrolyzed to Phe-tRNA bound is approximately 1 (Ravel *et al.*, 1970). Ribosome–poly(U)–AcPhe-tRNA complexes isolated in the absence of NH_4^+ and K^+ (ribosomes I) do not form AcPhe-puromycin at 30° without NH_4^+ or at 0° with NH_4^+. Similarly, these ribosomes do not form AcPhe-Phe-tRNA when interacted with complex II or 30° without NH_4^+ or at 0° with NH_4^+. The amount of Phe-tRNA from complex II bound to the ribosomes at 30° in the absence of NH_4^+ (as measured by retention of the ribosomes on a Millipore filter) is significantly less than that bound in the presence of NH_4^+. Also in the absence of NH_4^+ the binding of the Phe-tRNA to the ribosomes appears to be unstable; appreciable dissociation of the Phe-tRNA from the ribosomes occurs during centrifugation or gel filtration.

In contrast to the decrease in the amount of Phe-tRNA bound to ribosomes I at 30° in the absence of K^+ and NH_4^+, the amount of GTP from complex II hydrolyzed during the binding reaction is greatly increased, the ratio of GTP hydrolyzed to Phe-tRNA bound reaching as high as 8 to 1. If, as indicated above, Phe-tRNA readily dissociates from the ribosomes they may again interact with complex II, resulting in the hydrolysis of another GTP, and creating a high ratio of GTP hydrolyzed to Phe-tRNA recovered bound to the ribosomes. The ability of the ribosomes to form AcPhe-puromycin and AcPhe-Phe is rapidly restored when the ribosomes are heated with NH_4^+ and to a lesser extent with K^+. When the ribosomes are heated with NH_4^+, more Phe-tRNA is bound to the ribosomes, less GTP is hydrolyzed, and the ratio of GTP hydrolyzed to Phe-tRNA bound is restored to approximately 1.

Additional evidence for a relationship between the peptidyl transferase activity of the ribosomes and the interaction of the ribosomes with complex II was obtained with sparsomycin. Sparsomycin inhibits peptide-bond formation by inhibiting the peptidyl transferase activity of the 50S ribosomal subunit (Monro and Vazquez, 1967; Monro *et al.*, 1969). In the presence of NH_4^+, sparsomycin inhibits the formation of AcPhe-Phe as well as AcPhe-puromycin (Lucas-Lenard and Haenni, 1968). Incubation of the ribosomes with sparsomycin decreases the amount of Phe-tRNA from complex II bound to the ribosomes and increases the amount of GTP hydrolyzed, a result similar to that observed when the ribosomes are depleted of NH_4^+ and K^+. These data suggest a relationship between the peptidyl transferase activity of the ribosomes and the interaction of the ribosomes with complex II.

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