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Effect of Methanol on the Partial Reactions of Polypeptide Chain Elongation†

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ABSTRACT: The effect of methanol on a number of the partial reactions of protein synthesis with *Escherichia coli* ribosomes has been examined. The alcohol virtually abolished formation of *N*-Ac(Phe)$_2$-tRNA without producing a corresponding effect on *N*-acetylphenylalanylpuromycin formation or T-dependent binding of Phe-tRNA. T-dependent hydrolysis of GTP, on the other hand, was briskly stimulated. Similarly, methanol strongly inhibited translocation while stimulating the G-dependent reactions of [^{3}H]GTP binding to ribosomes and nonturnover hydrolysis of GTP. The effect of the alcohol on the G-catalyzed binding of [^{3}H]GDP and turnover hydrolysis of GTP was relatively minor. Ethanol, 2-propanol, and acetone were found to have effects qualitatively similar to those of methanol.

Methanol and ethanol are known to have a number of interesting effects on ribosomal structure and function. Monro and Marcker demonstrated that *Escherichia coli* ribosomes catalyzed an mRNA-independent formation of *N*-formylmethionylpuromycin from f-Met-tRNA or *N*-formylmethionyloligonucleotides in the presence of ethanol (Monro and Marcker, 1967) or methanol (Monro *et al.*, 1968). The reaction could be carried out by isolated 50S subunits (Monro, 1967a), and the replacement of puromycin by aminoacyl-tRNA resulted in the formation of dipeptidyl-tRNA and oligopeptidyl-tRNA (Monro, 1967b). Tompkins *et al.* (1970) observed a codon-independent termination reaction in the presence of ethanol, while Scolnick and Caskey (1969) found that the interaction of ribosome, terminator codon, and release factor was stabilized by ethanol. Tompkins (1970) was able to obtain a T-factor-dependent formation of dipeptide with codons as mRNA provided ethanol was present. We demonstrated that a highly specific extraction (the PI extraction) of the 50S subunit could be performed with ethanol and NH_4Cl under appropriate conditions (Hamel and Nakamoto, 1971; Hamel *et al.*, 1972). Ballesta *et al.* (1971) reported that the G-dependent guanosine triphosphatase activity of CsCl-extracted 50S particles could be reconstituted with methanol. These observations indicated that an examination of the effect of these alcohols on the G-dependent and T-dependent activities of *E. coli* ribosomes would be of interest. The results of our study with methanol are reported here.

Materials and Methods

Materials. [^{3}H]GDP of specific activity 1430 μCi/μmole was purchased from New England Nuclear. Fusidic acid was a gift of Dr. Josef Fried. Other materials were described previously (Hamel *et al.*, 1972).

Methods. E. coli NH_4Cl-washed ribosomes, protamine-treated supernatant, G and T factors, Phe-tRNA, and *N*-AcPhe-tRNA were prepared as described previously (Hamel *et al.*, 1972). The T factor used was the "peak 1T" factor repurified on hydroxylapatite (Hamel *et al.*, 1972). Protein concentrations were determined by the method of Lowry *et al.* (1951), with lysozyme as standard. All assays contained 0.01 M $MgCl_2$, 0.05 M imidazole-HCl (pH 7.4), 0.08 M NH_4Cl, and 0.012 M 2-mercaptoethanol, except as indicated. Polyphenylalanine synthesis assays were performed as described previously (Hamel *et al.*, 1972).

Preparation of the Ribosome–Poly(U)–N-AcPhe-tRNA Complex. The complex was prepared by a 1-hr incubation at 37° in a mixture containing 388 μg/ml of nonradioactive *N*-AcPhe-tRNA, 2 mg/ml of ribosomes, and 200 μg/ml of poly(U). After incubation the mixture was cooled to 0°.

Formation of N-Acetyl[^{14}C]phenylalanylpuromycin. Ribosome–poly(U)–*N*-AcPhe-tRNA complexes were formed as described above, except that 394 μg/ml of *N*-Ac[^{14}C]Phe-tRNA was used. Each assay tube contained, in a final volume of 0.250 ml, 0.125 ml of complex, 0.125 μmole of puromycin,

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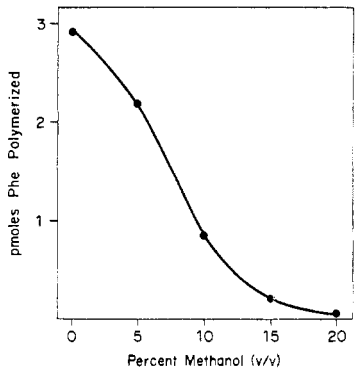

FIGURE 1: Effect of methanol on polyphenylalanine synthesis. Each reaction contained 10 μg of ribosomes, 0.015 ml of protamine-treated supernatant, 44 μg of tRNA charged with 28 pmoles of [^{14}C]phenylalanine, 10 μg of poly(U), 0.25 μmole of GTP, and methanol as indicated. Incubation was for 5 min at 37°.

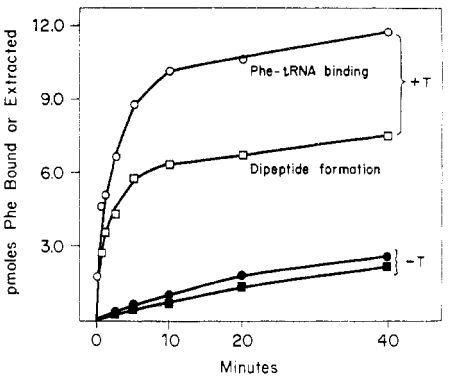

FIGURE 2: Kinetics of enzymatic binding and *N*-acetyldiphenylalanine formation. After formation of ribosome–poly(U)–non-radioactive-*N*-AcPhe-tRNA complexes (see Methods), 0.25 μmole of GTP, 54 μg of tRNA charged with 30 pmoles of [^{14}C]phenylalanine, and 4.1 μg of T factor were added where indicated. Incubation was for the indicated times at 0°. One set of reactions was stopped with binding buffer to measure enzymatic binding; and the other, with KOH, to measure dipeptide formation.

and methanol as indicated. Incubation was resumed for 0.5 min at 37°. The reactions were stopped with 1.0 ml of 0.1 M sodium acetate (pH 5.5), and the solutions were then extracted with 2.0 ml of ethyl acetate (Leder and Bursztyn, 1966). One milliliter of the ethyl acetate phase was counted in Bray's solution (Bray, 1960) in a Beckman scintillation counter.

Enzymatic Binding of [^{14}C]Phe-tRNA and Formation of N-Acetylphenylalanyl[^{14}C]phenylalanine. Each assay tube contained, in a volume of 0.250 ml, 0.125 ml of ribosome–poly(U)–*N*-AcPhe-tRNA complex, and [^{14}C]Phe-tRNA, GTP, T factor, and methanol, as indicated. Incubation was for the indicated times at 0° (Hamel *et al.*, 1972; Lucas-Lenard and Haenni, 1968). In binding assays the reactions were stopped and processed as described previously (Hamel *et al.*, 1972; Nirenberg and Leder, 1964). The Millipore filters were dried and counted either with a Nuclear Chicago low background counter or by scintillation in a toluene-based solution. Formation of *N*-acetylphenylalanyl[^{14}C]phenylalanine was measured by the method of Erbe *et al.* (1969): the reactions were stopped with 1.2 ml of 0.1 M KOH; the samples were then hydrolyzed at 37° for 30 min, acidified with 0.1 ml of concentrated HCl, and extracted with 2.0 ml of ethyl acetate. One milliliter of the ethyl acetate phase was counted in Bray's solution. The enzymatic binding and dipeptide formation studies were always performed simultaneously.

Translocation Assay. The initial stages were performed exactly as in the enzymatic binding and dipeptide formation assays. After addition of T factor, GTP, and [^{14}C]Phe-tRNA, the reaction mixtures were left at 0° for 1 hr, with no methanol at this stage. After 1 hr, the volume was increased to 0.40 ml with the addition of 0.125 μmole of puromycin, 0.15 μmole of GTP, 10 μg of G factor, and methanol as indicated. Incubation was resumed at 37° for 0.5 min. The reactions were stopped with 2.0 ml of 0.1 M sodium acetate (pH 5.5), and the samples were extracted with 3.0 ml of ethyl acetate. Two milliliters of the ethyl acetate phase was counted in Bray's solution.

G-Dependent Binding of [^{3}H]GDP and [^{3}H]GTP. Each assay tube contained, in a volume of 0.250 ml, 10 μg of G factor, 100 pmoles of [^{3}H]GDP or [^{3}H]GTP, 0.25 μmole of fusidic acid, and ribosomes and methanol as indicated. The reactions were stopped, and the samples were counted as described previously (Bodley *et al.*, 1970; Hamel *et al.*, 1972).

Guanosine Triphosphatase Assays. Specific components are described in the individual experiments. Reactions were stopped by addition of 1.0 ml of 5% trichloroacetic acid containing 2% charcoal (w/v) for adsorption of unreacted [γ-^{32}P]GTP, and the samples were processed as described previously (Erbe *et al.*, 1969; Hamel *et al.*, 1972).

Results

Effect of Methanol on Poly(phenylalanine) Synthesis. Figure 1 demonstrates the effect of methanol on poly(phenylalanine) synthesis. Increasing concentrations of the alcohol resulted in increasing inhibition of polypeptide synthesis, the inhibition being essentially complete at 15% methanol.

Effect of Methanol on T-Dependent Ribosomal Functions. As has been noted in several laboratories (Lucas-Lenard and Haenni, 1968; Ravel, 1967; Ravel *et al.*, 1969; Skoultchi *et al.*, 1969; Weissbach *et al.*, 1969) T factor catalyzes the binding of Phe-tRNA to a ribosome–poly(U)–*N*-acetyl-Phe-tRNA complex, resulting in the formation of *N*-Ac(Phe)$_2$-tRNA. Figure 2 presents the kinetics of this reaction at 0°. Binding of Phe-tRNA and formation of *N*-Ac(Phe)$_1$-tRNA occur in parallel. The extraction procedure used for measurement of dipeptide formation results in a virtually complete separation of phenylalanine and *N*-acetylphenylalanine between the aqueous and ethyl acetate phases (unpublished observations). Although diphenylalanine and larger oligomers could be partially extracted into the ethyl acetate phase (Pestka, 1969), the technique appears to be a reliable measure of *N*-acetyldiphenylalanine formation in this system: almost no radioactivity was extracted unless nonradioactive *N*-AcPhe-tRNA was included in the preincubation, and the material extracted cochromatographed with *N*-acetyldiphenylalanine (Hamel *et al.*, 1972; unpublished observations).

Methanol exhibited a differential effect on initial rates of enzymatic binding of Phe-tRNA and *N*-Ac(Phe)$_2$-tRNA formation (Figure 3A). Dipeptide formation was gradually inhibited with increasing concentrations of methanol, being almost completely inhibited in 20% methanol. Enzymatic binding, on the other hand, increased slightly up to 10% methanol and then decreased at higher concentrations with about a 45% inhibition in 20% methanol. This clearly suggests that the primary interference by methanol occurs after the binding of Phe-tRNA to the ribosome, but before the

TABLE I: Effect of Methanol on T-Dependent Guanosine Triphosphatase Activity.[a]

Condition	pmoles of GTP Cleaved		
	Experiment I		Experiment II
	No Methanol	With Methanol	With Methanol
1. Ribosomes only	0		0
2. T factor only	2.1	2.0	30
3. Ribosomes and T factor	2.5	35.6	390
4. Ribosomes, T factor, and poly(U)			310
5. Ribosomes, T factor, and Phe-tRNA	10.0		820
6. Ribosomes, T factor, Phe-tRNA, and poly(U)	29.0		640

[a] Each assay in experiment I contained, in a volume of 0.250 ml, 50 pmoles of [γ-^{32}P]GTP and the following, as indicated: 100 μg of ribosomes, 15.8 μg of T factor, 70 μg of nonradioactive Phe-tRNA, 25 μg of poly(U), and 20% methanol. Incubation was for 5 min at 37°. In experiment II, each assay contained, in a volume of 0.250 ml, 2.5 nmoles of [γ-^{32}P]GTP, 20% methanol (v/v), and the following, as indicated: 100 μg of ribosomes, 15.8 μg of T factor, 70 μg of nonradioactive Phe-tRNA, and 25 μg of poly(U). Incubation was for 5 min at 37°.

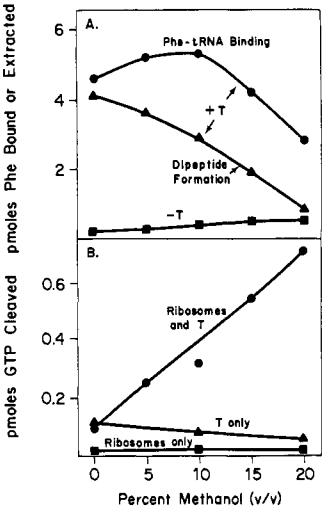

FIGURE 3: Effect of methanol on (A) enzymatic binding and *N*-acetyldiphenylalanine formation and (B) on T-dependent guanosine triphosphatase activity. (A) The assay was similar to that described in Figure 2, except that 44 μg of tRNA charged with 28 pmoles of [^{14}C]phenylalanine and methanol and 15.8 μg of T factor were added to each tube as indicated. Incubation was for 1 min at 0°. The reaction was initiated by adding the ribosomal complex to the other components. Methanol was present only in the 0° incubation. (B) Each assay contained, in a volume of 0.250 ml, 100 μg of ribosomes, 2.5 nmoles of [γ-^{32}P]GTP, 70 μg of nonradioactive Phe-tRNA, 15.8 μg of T factor, and methanol, as indicated. Incubation was for 5 min at 37°.

peptide bond is formed. No [^{14}C]Phe-tRNA was retained on the Millipore filter in the absence of ribosomes either with or without methanol (unpublished observations).

Since it has been shown that a T-dependent GTP hydrolysis must precede peptide bond formation in systems analogous to that used here (Haenni and Lucas-Lenard, 1968; Skoultchi *et al.*, 1970), we examined the effect of methanol on the cleavage of GTP. The uncoupled reaction, requiring ribosomes, T factor, and aminoacyl-tRNA, but not mRNA, was studied (Hamel *et al.*, 1972; Lin *et al.*, 1969; Ravel, 1967). The results in Figure 3B demonstrate that methanol strongly stimulates this reaction. The reaction in the presence and absence of methanol is compared in Table I. As was reported elsewhere (Hamel *et al.*, 1972), Phe-tRNA is required for the T-dependent hydrolysis of GTP in aqueous solution. This reaction is enhanced if poly(U) is present in addition to Phe-tRNA (Table I; Hamel *et al.*, 1972; Ono *et al.*, 1969), but poly(U) alone has no effect (Hamel *et al.*, 1972). In the presence of methanol, however, only T factor and ribosomes are required to obtain significant cleavage of GTP. Moreover, although Phe-tRNA continues to stimulate the reaction briskly, poly(U) has a slightly inhibitory effect even in the presence of Phe-tRNA.

Effect of Methanol on Peptide Bond Formation. Because the inhibition of dipeptide formation by methanol contrasted with its marked stimulation of GTP cleavage and its relatively minor effect on enzymatic binding, it was of interest to determine the effect of methanol on peptide bond formation with puromycin (Erbe *et al.*, 1969; Haenni and Lucas-Lenard, 1968; Traut and Monro, 1964). As can be seen in Figure 4, methanol inhibited formation of *N*-acetylphenylalanylpuromycin much less than it inhibited formation of *N*-Ac(Phe)$_2$-tRNA. This indicates that the primary effect of methanol formation is not on peptide formation *per se* (Monro, 1967a,b; Monro and Marcker, 1967; Monro *et al.*, 1968).

Effect of Methanol on G-Dependent Ribosomal Functions. G factor has long been implicated in translocation (Erbe *et al.*, 1969; Nishizuka and Lipmann, 1966; Pestka, 1968) and in a ribosome-dependent guanosine triphosphatase activity that can occur independently of polypeptide synthesis (Conway and Lipmann, 1964; Erbe *et al.*, 1969; Nishizuka and Lipmann, 1966). The effect of methanol on these two reactions was examined next. The effect of methanol on translocation, as measured by the sensitivity to puromycin of [^{14}C]Phe-tRNA bound enzymatically to the ribosome–poly(U)–*N*-AcPhe-tRNA complex, is shown in Figure 5A. Prior to reacting with puromycin, most of the bound [^{14}C]phenylalanine was presumably in the form of *N*-AcPhe[^{14}C]Phe-

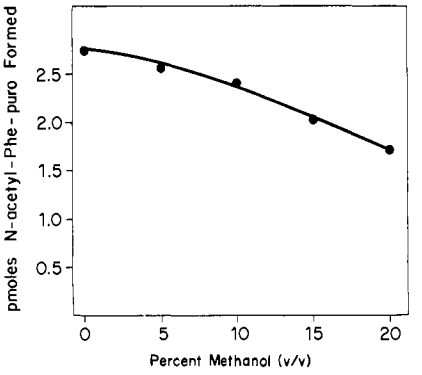

FIGURE 4: Effect of methanol on formation of *N*-acetylphenylalanylpuromycin. Ribosome–poly(U)–*N*-Ac[^{14}C]Phe-tRNA complexes were formed as described in the text. The indicated amount of methanol and 0.125 μmole of puromycin were added to each tube, and incubation was resumed for 0.5 min at 37°.

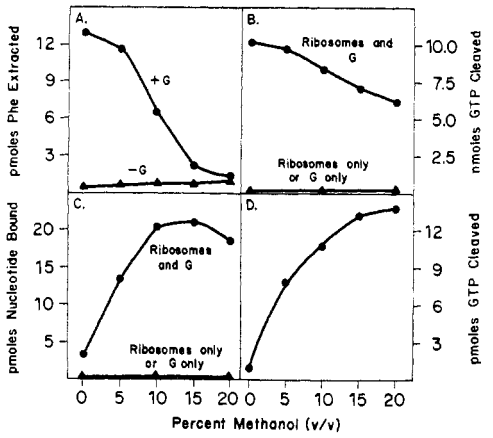

FIGURE 5: Effect of methanol on (A) translocation, (B) G-dependent guanosine triphosphatase activity, (C) G-dependent binding of [^{3}H]GTP to ribosomes, and (D) nonturnover G-dependent GTP cleavage. (A) Ribosome–poly(U)–nonradioactive-*N*-AcPhe-tRNA complexes formed in a 1-hr incubation at 37° were incubated for 1 hr at 0° after the addition of T factor, GTP, and [^{14}C]Phe-tRNA. Puromycin, GTP, and methanol, as indicated, were then added to each tube and the translocation reaction was initiated by adding G factor and incubating at 37° for 0.5 min. (B) Each assay contained, in a volume of 0.250 ml, 25 nmoles of [γ-^{32}P]GTP, 10 μg of G factor, 50 μg of ribosomes, and methanol, as indicated. Incubation was for 10 min at 37°. (C) Each assay contained 100 pmoles of [^{3}H]GTP, 0.25 μmole of fusidic acid, 0.01 M NH_4Cl, and methanol, 10 μg of G factor, and 100 μg of ribosomes as indicated. Incubation was for 1 min at 0° and was started by addition of G factor. (D) The conditions were the same as those described for C, except that [γ-^{32}P]GTP was used instead of [^{3}H]GTP, and GTP cleavage was measured instead of GTP binding, as described in the text.

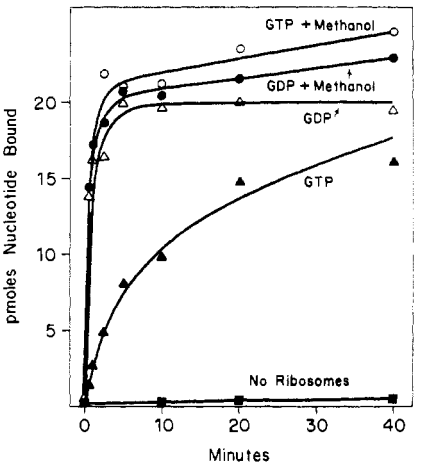

FIGURE 6: Kinetics of binding of [^{3}H]GDP and [^{3}H]GTP with and without methanol. Each assay contained 0.25 μmole of fusidic acid, 100 μg of ribosomes, 10 μg of G factor, and 0.01 M NH_4Cl as well as 100 pmoles of [^{3}H]GDP or [^{3}H]GTP and 20% methanol where indicated. Incubation was at 0°.

tRNA (see above, also Haenni and Lucas-Lenard, 1968; Lucas-Lenard and Haenni, 1968; Skoultchi *et al.*, 1969). Formation of puromycin derivatives was almost completely dependent on G factor, and the reaction was increasingly inhibited by higher concentrations of methanol with essentially complete inhibition in 20% methanol.

The effect of methanol on the G-dependent, ribosome-dependent guanosine triphosphatase activity (Conway and Lipmann, 1964; Erbe *et al.*, 1969; Nishizuka and Lipmann, 1966) is shown in Figure 5B. When measured under conditions in which each ribosome participated in many rounds of GTP cleavage (turnover cleavage), the effect of methanol was only mildly inhibitory. This is in sharp contrast both to its potent stimulation of T-dependent GTP cleavage (also under conditions in which each ribosome participated many times) and to its virtually complete inhibition of translocation at the higher concentrations.

We next examined the G-dependent binding of [^{3}H]GTP to ribosomes in the presence of fusidic acid (Bodley *et al.*, 1970; Brot *et al.*, 1971; Kaziro *et al.*, 1969). In contrast to its inhibition of translocation and its minimal effect on turnover GTP cleavage, methanol markedly stimulated the binding of [^{3}H]-GTP (Figure 5C). As in the absence of methanol, this binding was dependent on both ribosomes and G factor.

A number of investigators have demonstrated that the actual nucleotide bound to ribosomes is [^{3}H]GDP even when the nucleotide is added as GTP (Bodley *et al.*, 1970; Brot *et al.*, 1971; Kaziro *et al.*, 1969). The possibility that ribosome-bound [γ-^{32}P]GTP could be found in 20% methanol was examined, but no evidence was found for this. Instead, when GTP cleavage was examined under conditions employed in [^{3}H]GTP binding, methanol was found to stimulate cleavage as briskly as it did binding (Figure 5D).

The effect of methanol on the binding of [^{3}H]GDP was compared to its effect on the binding of [^{3}H]GTP (Table II). There were two notable differences in the binding of the two nucleotides: much more [^{3}H]GDP was bound than [^{3}H]GTP in the absence of methanol, and methanol stimulated [^{3}H]-GDP binding only minimally in comparison to its strong stimulation of [^{3}H]GTP binding. In the absence of fusidic acid, methanol alone induced significant binding of both [^{3}H]GDP and [^{3}H]GTP, and in about the same amounts.

In the course of these studies it was observed that the binding of [^{3}H]GTP was a relatively slow reaction. In Figure 6, the rates of binding of [^{3}H]GDP and [^{3}H]GTP in both the presence and absence of methanol are compared. Once again the minimal effect of methanol on [^{3}H]GDP binding is in sharp contrast to its great acceleration of [^{3}H]GTP binding. It is also noteworthy that [^{3}H]GDP is bound much more rapidly than [^{3}H]GTP in the absence of methanol, although equivalent amounts of nucleotides appear to be eventually bound in both instances.

TABLE II: Effect of Methanol and Fusidic Acid on the G-Dependent Binding of Guanine Nucleotides.[a]

Condition	pmoles of Nucleotide Bound	
	[^{3}H]GTP	[^{3}H]GDP
1. Complete	16.8	19.1
2. − fusidic acid	6.0	6.7
3. − methanol	2.4	16.2
4. − methanol, − fusidic acid	0.4	2.0
5. − G factor	0.3	0.1
6. − ribosomes	0.4	0.1

[a] Each assay contained 0.01 M NH_4Cl and the following, as indicated: 100 pmoles of [^{3}H]GTP or [^{3}H]GDP, 100 μg of ribosomes, 10 μg of G factor, 0.25 μmole of fusidic acid, and 20% methanol (v/v). The incubation was started by addition of G factor and continued for 1 min at 0°.

Effect of Other Organic Solvents. To gain some insight into the basis for the effect of methanol on the various partial reactions, the effects of ethanol, 2-propanol, and acetone were compared to that of methanol in the ribosomal activities briskly stimulated by the latter (Table III). All three solvents were found to stimulate briskly both the T-dependent guanosine triphosphatase activity (experiment I) and the binding of [^{3}H]GTP to ribosomes (experiment II). The relative effect of the solvents, however, differed in the two assays. In the T-dependent guanosine triphosphatase activity their order of stimulation was methanol > acetone > ethanol > 2-propanol, while in the G-dependent [^{3}H]GTP binding activity their order was 2-propanol > ethanol > methanol > acetone. Stimulation of nonturnover GTP cleavage was observed along with the stimulation of [^{3}H]GTP binding with all the solvents (unpublished observations).

TABLE III: Effect of Various Organic Solvents on T-Dependent Guanosine Triphosphatase and G-Dependent [^{3}H]GTP Binding Activities.[a]

Solvent Added	Experiment I T-Dependent Guanosine Triphosphatase (pmoles of GTP Cleaved)	Experiment II G-Dependent Binding (pmoles of Nucleotide Bound)
1. None	10	3.1
2. Methanol	660	10.5
3. Ethanol	310	12.3
4. 2-Propanol	110	13.0
5. Acetone	470	7.5

[a] Each assay in experiment I contained, in a volume of 0.250 ml, 100 μg of ribosomes, 15.8 μg of T factor, 2.5 nmoles of [γ-^{32}P]GTP, 70 μg of nonradioactive Phe-tRNA, and 20% of the indicated organic solvent (v/v). Incubation was for 5 min at 37°. All values have been corrected for the amount of hydrolysis with T factor alone. In experiment II, each assay contained 100 μg of ribosomes, 10 μg of G factor, 100 pmoles of [^{3}H]GTP, 0.25 μmole of fusidic acid, 0.01 M NH_4Cl, and 5% of the indicated organic solvent. Incubation was for one min at 0° and was started by addition of G factor.

Discussion

Effect of Methanol on T-Dependent Functions. Methanol was shown here to be an effective inhibitor of *N*-Ac(Phe)$_2$-tRNA formation, although Phe-tRNA binding was only partially inhibited at the higher methanol concentrations, and peptide bond formation, as measured by *N*-acetylphenylalanylpuromycin formation, was only minimally affected. The results are somewhat similar to those obtained in the binding reaction with the GTP analog, GDPCP (Haenni and Lucas-Lenard, 1968; Skoultchi *et al.*, 1970), Phe-tRNA binding occurring in both instances without the formation of a peptide bond. A significant difference, however, exists between the two cases in that no peptide bond was formed in the presence of methanol despite an accelerated hydrolysis of GTP. Methanol, in other words, appears to inhibit *N*-Ac(Phe)$_2$-tRNA formation by interfering with the effective utilization of GTP.

An interesting effect of methanol on the T-dependent guanosine triphosphatase activity was the alteration in the requirements of the reaction. In the presence of methanol, Phe-tRNA no longer was an obligatory component in the reaction mixture, cleavage of GTP occurring with only ribosomes and T factor; and poly(U) no longer had a stimulatory effect, but was, in fact, slightly inhibitory.

It is of interest to note that the requirement for poly(U) in the T-dependent guanosine triphosphatase reaction varied in several systems studied. Poly(U) was only stimulatory for the reaction in our system (Hamel *et al.*, 1972), was essential in the system with *Bacillus stearothermophilus* factors (Ono *et al.*, 1969), and was variable in its effect in the reticulocyte system (Lin *et al.*, 1969).

Effect of Methanol on G-Dependent Functions. Like dipeptide formation, translocation was strongly inhibited by increasing methanol concentrations. G-dependent binding of [^{3}H]GTP to ribosomes and nonturnover G-dependent cleavage, on the other hand, were strongly stimulated, while binding of [^{3}H]GDP (in the presence of fusidic acid) and turnover GTP cleavage were not significantly affected by methanol.

The different effect of methanol on nonturnover and turnover cleavage of GTP was not due to the different conditions employed in the two reactions since similar results were obtained when the experimental conditions were modified (unpublished observations). A more likely explanation is that while methanol stimulates cleavage of GTP, it interferes to some degree with the recovery of the G–ribosome system for further hydrolysis, and the turnover rate reflects the net result of these two effects. Interference with the recovery of the G–ribosome system is suggested by the observation that methanol stimulates binding of guanine nucleotides to ribosomes. This effect of methanol is reminiscent of that of fusidic acid on the G-dependent binding of guanine nucleotides (Bodley *et al.*, 1970; Brot *et al.*, 1971; Kaziro *et al.*, 1969; Tanaka *et al.*, 1968). It has been suggested that fusidic acid stimulates the binding reaction by stabilizing the G–GDP–ribosome complex, which normally dissociates rapidly and frees G for participation in a new round of GTP hydrolysis. Unlike methanol, however, fusidic acid is a potent inhibitor of turnover GTP hydrolysis. This may be due to differences in the stability of the G–GDP–ribosome complex in the presence of the two reagents, as well as to the stimulation of nonturnover GTP cleavage by methanol.

Although inhibition of translocation with minimal effect on turnover GTP hydrolysis has been described for a number of antibiotics (Pestka and Brot, 1971), the effect of methanol in stimulating nonturnover GTP cleavage is thus far unique. Again, as in the T-factor-dependent activities, methanol appears to interfere with the effective utilization of GTP; the functionally significant activity, translocation, is severely inhibited while nonturnover GTP cleavage is briskly stimulated.

Binding of [^{3}H]GTP and [^{3}H]GDP to Ribosomes. Baliga and Munro (1971) recently described an interconversion of mammalian ribosomes from a form binding GTP slowly, to a form binding GTP rapidly, the binding reactions requiring transferase II. This change was attributed to conversion of ribosomes bearing peptidyl-tRNA in the A site (slow binding) to ribosomes binding peptidyl-tRNA in the P site (rapid binding). To determine whether the differential rate of nucleotide binding we observed with [^{3}H]GTP and [^{3}H]GDP was a manifestation of this same phenomenon, we attempted unsuccessfully to increase the rate of [^{3}H]GTP binding by a number of manipulations of the poly(U) model system. Instead of

enhancing the slow [^{3}H]GTP binding, incubation of the ribosomes with poly(U) and *N*-AcPhe-tRNA resulted in a substantial reduction in the binding of both nucleotides with little effect on the relative rates of binding (unpublished observations). We have thus far been able to accelerate the relative rate of [^{3}H]GTP binding only with organic solvents.

Effect of Other Organic Solvents. The studies with ethanol, 2-propanol, and acetone rule out methanolysis as the primary mechanism for the stimulation of the T- and G-dependent reactions by methanol. The order of effectiveness of the four solvents tested, including methanol, did not coincide with the order of nucleophilicity of these solvents (Kirby and Varvoglis, 1967). The stimulation may be due to a solvent effect, perhaps involving a change in ribosomal conformation.

Finally, the four solvents used in our study were also found to be effective by Monro *et al.* (1969) in stimulating the codon-independent peptidyl transferase activity. The order of effectiveness of the solvents differs, however, in the peptidyl transferase and the T- and G-dependent guanosine triphosphatase activities.

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