Ribosomal Activities Dependent on Elongation Factors T and G. Effects of Methanol†

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ABSTRACT: Characteristics and requirements of the elongation factor T (EF T) and elongation factor G (EF G) dependent GTPase are altered in the presence of 20% (v/v) methanol. This alcohol increases the NH_4^+ concentration and decreases the Mg^{2+} concentration required for maximum hydrolysis dependent on EF T. Concerning EF G dependent GTP hydrolysis, optimal Mg^{2+} concentration is decreased in the presence of methanol and the reaction is made NH_4^+ independent by the alcohol. Although methanol uncouples EF T dependent GTP hydrolysis, from the aminoacyl-tRNA binding the coupled GTPase activity also takes place in the presence of alcohol. Aminoacyl-tRNA does not affect the EF T dependent GTP hydrolysis in the presence of methanol, but it inhibits EF G dependent GTPase. Methanol enhances aminoacyl-tRNA binding to ribosomes at low Mg^{2+} concentrations (under 5 mM) but inhibits this binding at higher Mg^{2+} concentrations (over 10 mM).

Important advances have been achieved recently in the understanding of the relationship between ribosomal structure and function. These studies were mainly based on reconstitution of the ribosomal structure starting from its individual components. This type of study has allowed the identification of a number of ribosomal proteins involved in some of the ribosomal functions (for a review, see Kurland, 1972).

Interesting data can also be obtained using other approaches such as changing the solvent composition to induce conformational changes in the ribosomal structure (Petermann *et al.*, 1972). The solvent certainly affects the functions of ribosomes since it has been shown that the presence of methanol or ethanol affects the peptidyltransferase activity (Monro and Marcker, 1967; Monro *et al.*, 1968, 1969), the polypeptide chain termination (Tompkins *et al.*, 1970; Scolnick and Caskey, 1969), and elongation factors dependent activities of the ribosomes (Ballesta and Vazquez, 1972a; Hamel and Nakamoto, 1972). It is obvious that the proper interpretation of these findings might provide more understanding of the structure of ribosomes.

EF T[1] catalyzes binding of aminoacyl-tRNA to the ribosomes through the formation of a ternary complex (aminoacyl-tRNA·EF T·GTP) that interacts with the ribosome·mRNA complex (for a review, see Lucas-Lenard and Lipmann, 1971). As a result of this interaction aminoacyl-tRNA becomes bound and a molecule of GTP is hydrolyzed (for a review, see Lucas-Lenard and Lipmann, 1971). This GTP hydrolysis is obviously dependent on the presence of aminoacyl-tRNA and mRNA. However when methanol is present in the reaction a strong GTPase activity is observed dependent only on the presence of ribosomes and EF T (Ballesta and Vazquez, 1972a; Hamel and Nakamoto, 1972). This activity is uncoupled from the aminoacyl-tRNA binding and recalls the ribosome and EF G dependent GTP hydrolysis uncoupled from translocation (Nishizuka and Lipmann, 1966).

Since there is still the alternative of the existence of one or more sites on the 50S ribosome subunit implicated on the different GTPase activities, we have considered that a comparative study of the effect of methanol on EF T and EF G dependent GTPase activities would be of interest. We have carried out these studies and the results obtained are presented in this contribution.

Materials and Methods

Materials. [γ-^{32}P]GTP was prepared following the method described by Glynn and Chapell (1964). Purified $tRNA^{Phe}$ (a gift from Dr. G. D. Novelli, Oak Ridge Laboratories) and commercial deacylated tRNA from *Escherichia coli* (either from General Biochemicals or Schwarz) were charged with ^{14}C-labeled phenylalanine (513 Ci/mol) (the Radiochemical Centre, Amersham). Ribosomes were prepared from *E. coli* MRE 600 or D-10 as previously indicated (Ballesta and Vazquez, 1972); 1 A_{260} unit = 24.5 pmol of 70S ribosomes. Electrophoretically pure elongation factors EF G and EF T (T_u + T_s) were obtained according to Parmeggiani *et al.* (1971).

Methods used in the present report have been previously described (Ballesta and Vazquez, 1972; Ballesta, 1973). The guanosine triphosphatase assay was carried out following the procedure of Conway and Lipmann (1964) as described by Modolell and Vazquez (1973). Unless otherwise described in the text, buffer and ion concentrations in the reaction mixtures were 80 mM NH_4Cl, 10 mM $Mg(OAc)_2$, 10 mM Tris-HCl (pH 7.8), and 1 mM dithiothreitol. The order of addition of the reaction components was: buffer, ribosomes, poly(U), aminoacyl-tRNA, either EF T or EF G, and methanol. Finally reactions were initiated by the addition of [γ-^{32}P]GTP. The concentration of each component was as indicated in the text.

Binding of Phe-tRNA to ribosomes was measured by the Millipore filtration procedure (Nirenberg and Leder, 1964) using conditions similar to those described for the GTPase assays.

Results

EF T, Ribosomes, and GTP Requirements. The EF T dependent hydrolysis of GTP is uncoupled from the binding of the ternary complex aminoacyl-tRNA·EF T_u·GTP to the ribosome·mRNA complex in the presence of 20% methanol.

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[1] Abbreviations used are: EF T, elongation factor T (Tu + Ts); EF G, elongation factor G.

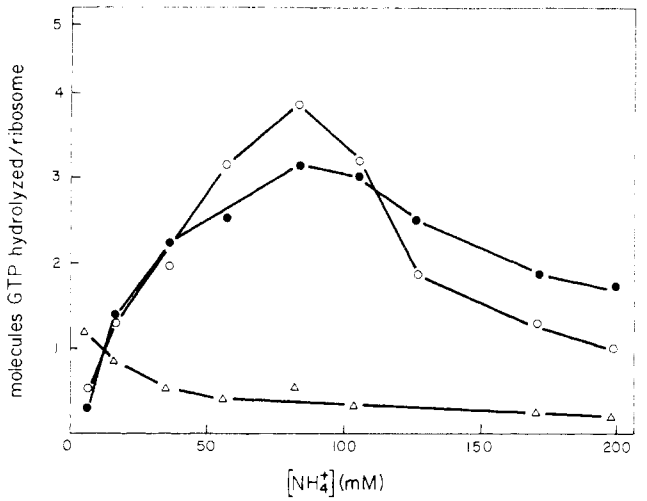

FIGURE 1: Dependence of EF T dependent GTPase on NH_4^+ concentration. Compositions of the reaction mixtures were: (1) (●—●) ribosomes, EF T, Phe-tRNA, poly(U), and methanol; (2) (△—△) as (1) minus methanol; (3) (○- - -○) as (1) minus Phe-tRNA and poly(U). Conditions described in Methods: 0.67 μM ribosome, 0.016 mg/ml of EF T, 0.33 μM Phe-tRNA, 0.1 mg/ml of poly(U), 1.4 μM [γ-^{32}P]GTP, and 20% v/v methanol.

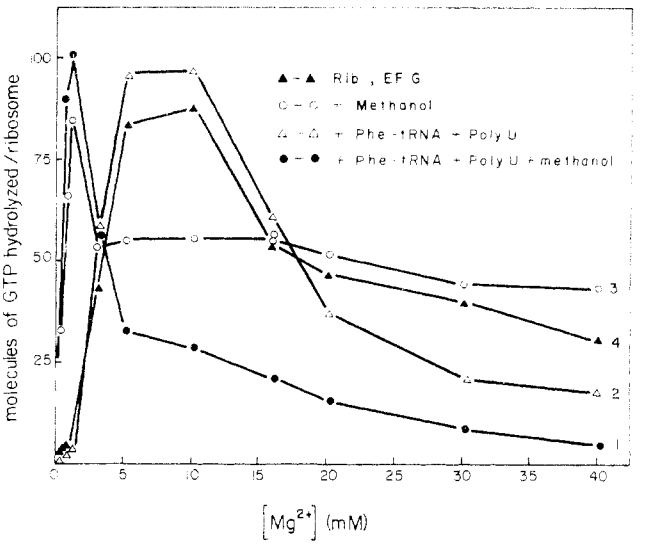

FIGURE 3: Effect of Mg^{2+} concentration on EF G dependent GTPase. Compositions of the reaction mixtures were: (1) (●—●) 0.29 μM ribosomes, 0.045 mg/ml of EF G, 0.151 μM Phe-tRNA, 0.1 mg/ml of poly(U), 103.3 μM [γ-^{32}P]GTP, and 20% (v/v) methanol; (2) (△—△) as (1) minus methanol; (3) (○—○) as (1) minus Phe-tRNA and poly(U); (4) (▲—▲) as (1) minus Phe-tRNA, poly(U), and methanol.

In these conditions EF T and ribosomes but not poly(U) and Phe-tRNA are required for GTP hydrolysis (Ballesta and Vazquez, 1972a). In this paper we will refer to this reaction as the uncoupled EF T dependent GTPase. The reaction is linearly dependent on the concentration of ribosomes and EF T, and the rate of GTP hydrolysis as a function of GTP concentration displays a typical hyperbolic curve of the Michaelis–Menten form from which a K_m for GTP of 1×10^{-6} M is deduced (data not shown).

Optimal Ammonium Ion Requirement for EF T and EF G Dependent GTP Hydrolysis. In the presence of methanol there is a maximum for uncoupled EF T dependent GTP hydrolysis at 80 mM ammonium ion concentration. The ammonium ion concentration required for maximal activity is not affected by the presence of Phe-tRNA and poly(U) (Figure 1). However, in the absence of alcohol maximal EF T dependent GTP hydrolysis takes place at very low ammonium ion concentration (Figure 1) in accordance with previous data reported by other workers (Ravel *et al.*, 1970). Although in this case Phe-tRNA and poly(U) are present in the reaction mixture the GTP hydrolysis that takes place at low NH_4^+ is also partially uncoupled since very low Phe-tRNA binding occurs under the ionic conditions used (Ravel *et al.*, 1970).

GTP hydrolysis catalyzed by EF G and ribosomes is maximal at 80 mM ammonium concentration (Figure 2) but monovalent ions are not required for EF G dependent GTP hydrolysis when 20% (v/v) of methanol is present. In fact, at optimum

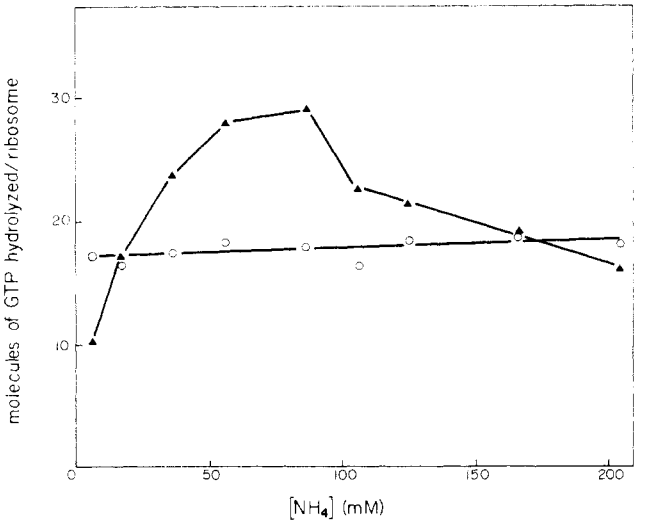

FIGURE 2: Effect of NH_4^+ concentration on EF G dependent GTPase. Compositions of the reaction mixtures were: (1) (▲—▲) ribosomes, EF G, minus methanol; (2) (○- - - -○) as (1) plus methanol. Conditions and concentrations as in Figure 1 except that EF G concentration was 0.021 mg/ml and [γ-^{32}P]GTP was added to a final concentration 41 μM.

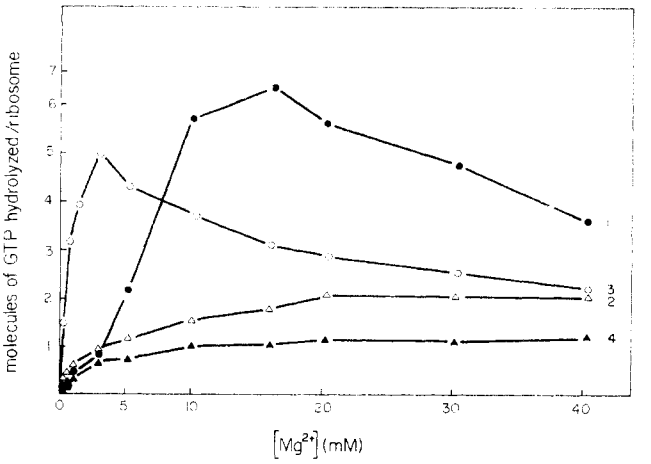

FIGURE 4: Effect of Mg^{2+} concentration on EF T dependent GTPase. Compositions of the reaction mixtures were: (1) (●—●) 0.29 μM ribosomes, 0.033 mg/ml of EF T, 0.151 μM Phe-tRNA, 0.1 mg/ml of poly(U), 6.7 μM [γ-^{32}P]GTP, and 20% (v/v) methanol; (2) (△—△) as (1) minus methanol; (3) (○- - -○) as (1) minus Phe-tRNA and poly(U); (4) (▲—▲) as (1) minus Phe-tRNA, poly(U), and methanol.

TABLE I: Inhibition by Thiostrepton of EF T Dependent GTP Hydrolysis in the Absence of Methanol.[a]

Conditions	Thiostrepton Concn (M)	pmol of GTP Hydrolyzed 3 mM Mg^{2+}	10 mM Mg^{2+}	30 mM Mg^{2+}
Complete	10^{-5}	3.2	8.0	15.9
Complete		8.1	15.8	32.7
− Phe-tRNA and poly(U)	10^{-5}	7.3	6.8	12.5
− Phe-tRNA and poly(U)		6.9	7.6	11.8

[a] The reaction was carried out in the standard ionic conditions at 30° for 3 min; 0.05 ml of the reaction mixtures contained 0.278 μM *E. coli* D-10 ribosomes, 0.021 mg/ml of EF T, 0.21 μM of Phe-tRNA, 0.1 mg/ml of poly(U), and 2 μM [γ-^{32}P]GTP.

NH_4^+ concentration there is a noticeable inhibition of this activity by methanol.

Effect of Magnesium Ion Concentration on EF G Dependent GTP Hydrolysis. The effect of magnesium ion concentration on the GTP hydrolysis dependent on EF G is shown in Figure 3. Maximum activity was observed at 8 mM magnesium concentration in the absence of methanol. This maximum was independent of the presence or absence of aminoacyl-tRNA and mRNA. When methanol was present the optimum Mg^{2+} concentration was lowered to 1–2 mM. In both cases it is interesting to note that, in agreement with previous results from our laboratory (Modolell and Vazquez, 1973), the EF G dependent GTPase was inhibited at high magnesium concentration when aminoacyl-tRNA and mRNA are present. However, at low magnesium concentrations, we found some stimulation by these two components, that might correspond to an unspecific effect similar to the one reported by Nishizuka and Lipmann (1966) by different RNA preparations.

Magnesium Ion Requirement for the EF T Dependent GTP Hydrolysis. The results obtained studying the effect of magnesium ion on EF T dependent GTP hydrolysis are shown in Figure 4. The uncoupled EF T dependent GTPase that takes place in the presence of EF T, ribosomes, and methanol displayed a maximum activity at 3–4 mM concentration of magnesium ion. This maximum is displaced to higher magnesium concentrations by the presence of aminoacyl-tRNA and poly(U). However contrary to what is found with EF G dependent GTPase (Figure 3), we have not detected any inhibition of the uncoupled GTP hydrolysis induced by methanol and dependent on EF T by the presence of aminoacyl-tRNA and poly(U) even at very high concentrations of magnesium ion.

In the absence of methanol the GTP hydrolysis taking place in the presence of aminoacyl-tRNA and mRNA (coupled GTPase) was also dependent on magnesium ion concentration but we were not able to determine the optimal concentration. This was probably due to the stimulation that increasing concentrations of magnesium caused in the GTP hydrolysis taking place in the absence of aminoacyl-tRNA and poly(U) (Figure 4). In order to see whether this uncoupled methanol-independent GTP hydrolysis had characteristics similar to the methanol-induced activity, we tested its sensitivity to thiostrepton which is known to inhibit the GTP hydrolysis coupled

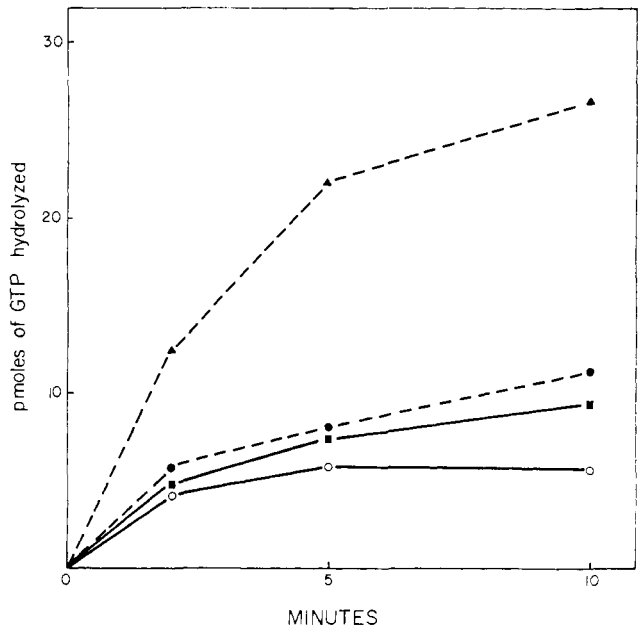

FIGURE 5: Effect of ribosome concentration of EF T dependent GTP hydrolysis. 0.05-ml reaction mixtures contained 0.028 mg/ml of EF T, 0.114 μM Phe-tRNA, 0.1 mg/ml of poly(U), and 1.05 μM [γ-^{32}P]GTP. Other components added to the reaction mixtures were: (○—○) 0.15 μM ribosomes; (■—■) 0.15 μM ribosomes and 20% methanol; (●- - - -●) 0.6 μM ribosomes; (▲- - - -▲) 0.6 μM ribosomes and 20% methanol.

to the aminoacyl-tRNA binding to the ribosomes (Modolell *et al.*, 1971) but which does not interfere with the methanol-induced uncoupled GTPase (Ballesta and Vazquez, 1972a). As it is shown in Table I the antibiotic inhibited by about 50% the GTPase in the presence of phenylalanyl-tRNA and poly(U) but did not affect the uncoupled GTPase activity.

Effect of Ribosome Concentration on EF T Dependent GTP Hydrolysis. We have studied the effect of the ribosome concentration on the rate and extent of the GTP hydrolysis taking place in the presence or in the absence of methanol at 5 mM Mg^{2+} (Figure 5). At low ribosome concentrations there was practically no effect of the alcohol on the initial rate of GTP hydrolysis although the total extent of the reactions was enhanced. At high ribosome concentration both the rate and the extent of the reaction were strongly stimulated by methanol. These results confirm our previous data (Ballesta and Vazquez, 1972a) and suggests that the alcohol does not interfere with the coupled hydrolysis of GTP, which apparently takes place normally, but strongly stimulates the uncoupled reaction carried out by the excess of ribosomes. This conclusion is strengthened by the results obtained studying the effect of thiostrepton on EF T dependent GTPase at different ribosome concentrations (Table II). Increasing concentrations of ribosomes decreased the inhibition caused by the antibiotic.

Effect of Phe-tRNA on EF T and EF G Dependent GTP Hydrolysis. Since we had observed a displacement by Phe-tRNA of the optimum requirement of magnesium ions for EF T dependent uncoupled GTPase (Figure 4), we also studied the effect of phenylalanyl-tRNA concentration on the activity at the two optimal Mg^{2+} concentrations. We found that the extent of either inhibition at 3 mM Mg^{2+} or stimulation at 12 mM Mg^{2+} was dependent on the tRNA concentration (data not shown). However, this effect seems not to be due specifically to the poly(U)-directed binding of Phe-tRNA to the ribosome, since it was also observed when deacylated tRNA

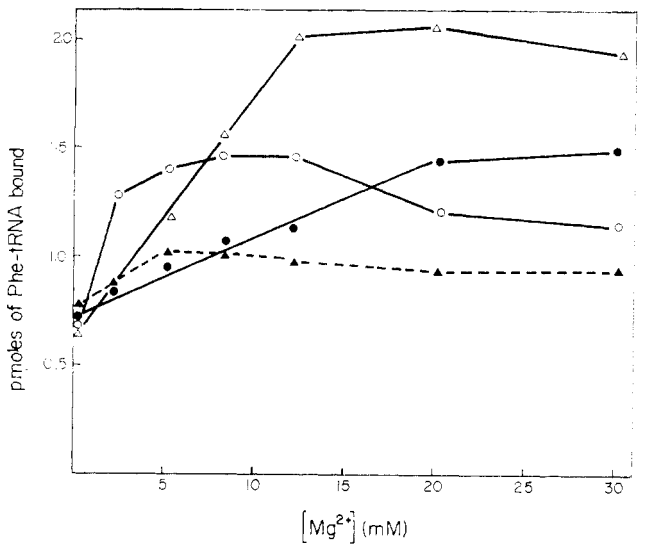

FIGURE 6: Effect of Mg^{2+} concentration on Phe-tRNA binding to the ribosomes. (1) (△—△) Enzymic binding; (2) (○—○) enzymic binding in the presence of 20% v/v methanol; (3) (●—●) nonenzymic binding; (4) (▲—▲) nonenzymic binding in the presence of 20% methanol. Conditions as described under Materials and Methods.

was used either in the presence or in the absence of poly(U) (Table III).

It is clear from the results described above that the nonspecific effect of aminoacylated bulk tRNA on the GTPase will mask the possible inhibition due to the specific binding of aminoacyl-tRNA which otherwise might be similar to that observed in the case of EF G dependent GTPase (Figure 3 and Modolell and Vazquez, 1973).

To overcome this difficulty we performed similar experiments using purified $tRNA^{Phe}$. In order to minimize the unspecific effect of the tRNA, the activity of samples preincubated with Phe-$tRNA^{Phe}$ at 20 mM Mg^{2+} in order to obtain maximum binding was compared with the activity of samples in which Phe-tRNA added at the same time as [γ-^{32}P]GTP (Table IV). If the bound tRNA has any effect on the GTPase activity this has to be preferentially expressed in the initial rate of reaction of the preincubated samples. As expected we found a noticeable inhibition of the rate of EF G dependent GTP hydrolysis in the samples at 20 mM Mg^{2+}

TABLE II: Effect of Ribosome Concentration on the Inhibition by Thiostrepton of EF T Dependent GTP Hydrolysis in the Presence of Methanol.[a]

	pmol of GTP Hydrolyzed		
μM Ribosome Concn	− Thiostrepton	+ Thiostrepton	% Inhibn
0.08	14.2	7.1	50
0.16	14.7	10.1	31.3
0.24	15.8	12.9	19.6
0.32	17.6	16.1	9.6

[a] The reaction mixture contained in 0.05 ml of standard buffer: ribosomes as indicated above, 0.018 mg/ml of EF T, 0.108 μM Phe-tRNA, 0.1 mg/ml of poly(U), 10 mM Mg Ac, 2 μM [γ-^{32}P]GTP, and 20% (v/v) of methanol. Incubations were carried out at 30° for 3 min.

TABLE III: Effect of Poly(U) and Deacylated tRNA on Methanol-Uncoupled EF T Dependent GTP Hydrolysis.[a]

			pmol of GTP Hydrolyzed			
			+ Poly(U)		− Poly(U)	
tRNA	Phe-tRNA (μM)	Total tRNA (mg/ml)	3 mM Mg^{2+}	12 mM Mg^{2+}	3 mM Mg^{2+}	12 mM Mg^{2+}
Phe-tRNA	0.2	0.45	23.4	88.5	21.3	78.9
Deacylated tRNA		0.35	28.6	56.1	23.7	65.4
None			47.8	42.5	43.9	43.2

[a] Concentrations of the reaction components in the standard ionic conditions were 0.21 μM ribosomes, 0.020 mg/ml of EF T, 0.1 mg/ml of poly(U), 3.3 μM [γ-^{32}P]GTP, and 20% (v/v) methanol.

but not in those preincubated at 3 mM, since at low Mg^{2+} concentration the extent of Phe-$tRNA^{Phe}$ binding is very small. On the other hand, the rate of hydrolysis dependent on EF T was practically unaffected by the preincubation with Phe-$tRNA^{Phe}$ at both of the Mg^{2+} concentrations tested.

Effect of Magnesium Ions on Phe-tRNA Binding to Ribosomes. It has been previously reported that methanol had a small effect on the aminoacyl-tRNA binding to ribosomes (Ballesta and Vazquez, 1972a; Hamel and Nakamoto, 1972). However since we have observed in this work a dramatic effect of the alcohol in the ionic requirements of the GTPase activity, we have reinvestigated this problem. In Figure 6 we represent the extent of enzymic and nonenzymic binding of Phe-tRNA in the presence and in the absence of 20% methanol, as a function of the Mg^{2+} concentration. In the absence of methanol we found an increase in the binding of Phe-tRNA at higher magnesium concentration as also observed by other

TABLE IV: Inhibition of GTP Hydrolysis by Phe-$tRNA^{Phe}$.[a]

		pmol of GTP Hydrolyzed			
		EF G		EF T	
Conditions		3 mM Mg^{2+}	20 mM Mg^{2+}	3 mM Mg^{2+}	20 mM Mg^{2+}
Preincubation	Incubation				
Phe-$tRNA^{Phe}$		18.1	8.0	13.2	10.4
	Phe-$tRNA^{Phe}$	18.3	12.7	11.8	9.3

[a] Reaction mixtures containing ribosomes, poly(U), and Phe-$tRNA^{Phe}$ when required, in 0.025 ml, were incubated at 30° for 10 min, cooled on ice, followed by addition of 0.01 ml of methanol and 0.015 ml of a mixture containing the required elongation factor (either EF T or EF G), [γ-^{32}P]GTP, and Phe-$tRNA^{Phe}$ when indicated. A second incubation was then carried out for 1 min at 0°. Through all the experiment the ionic conditions were 80 mM NH_4^+, 20 mM Tris-HCl (pH 7.8), and 1 mM dithiothreitol. The final concentrations of the other components were: 0.16 μM *E. coli* MRE 600 ribosomes, 0.128 μM Phe-$tRNA^{Phe}$, 0.023 mg/ml of EF G, 0.022 mg/ml of EF T, and 2 μM [γ-^{32}P]GTP.

workers (Ravel, 1967). When the nonenzymic binding is subtracted from the enzymic one, an optimum for enzymic binding was found at 12 mM Mg^{2+} concentration. When methanol was present we found a stimulation of the binding at low Mg^{2+} concentrations and a significant inhibition at higher concentrations.

Discussion

The effect of alcohols on ribosomal structure has been studied by several authors (Spirin and Lishnevskaya, 1971; Petermann *et al.*, 1972). Ethanol, probably due to its cohesive effect on hydrogen bonding, strengthens the ribosomal structure increasing the sedimentation coefficient and stabilizing the association of the subunits (Petermann *et al.*, 1972). Indeed one of the effects of methanol in our experiments was to lower the Mg^{2+} requirements for some of the activities tested, such as EF G dependent GTPase (Figure 3) and aminoacyl-tRNA binding (Figure 6). On the other hand, increasing Mg^{2+} concentrations could mimic the effect of methanol, stimulating the uncoupled EF T dependent GTP hydrolysis. This is in agreement with the stabilizing role of methanol since Mg^{2+} ions are supposed to have the same effect (Gesteland, 1966).

The monovalent ions seem to have an opposite effect to the Mg^{2+} ions on ribosome structure. High concentrations of monovalent ion are known to release proteins from the ribosome indicating that they have a loosening effect on the ribosomal structure. From a functional point of view it looks as if there is an optimum ratio of the two types of ions and increasing concentrations of one of them has to be compensated by increasing the other also. The effect of methanol on the ammonium ion requirements of the GTPase activities is in agreement with this idea. For instance, in the presence of alcohol the optimum ammonium ion concentration for the EF T dependent GTPase was displaced to higher values (Figure 1). The methanol effect in the case of EF G dependent activity is more difficult to interpret since it apparently makes the reaction ammonium ion independent (Figure 3). However, it is possible that the presence of methanol displaces the requirement for ammonium ions to concentrations even higher than those used in our experiments.

If we compare the ionic conditions required for optimal EF G and EF T dependent uncoupled GTPase it is clear that either in the presence or in the absence of methanol, a higher magnesium concentration (or a lower ammonium) is needed for the EF T dependent activity suggesting that a more compact structure is required for this activity to be expressed. This might perhaps indicate that two or more sites of the ribosome, normally accessible to EF T only when present as the ternary complex aminoacyl-tRNA·EF T·GTP, have to be brought close enough together to be reached by EF T alone in order that the uncoupled EF T dependent GTP hydrolysis shall take place.

It appears that in general the conformational changes induced by the alcohol in the ribosomal structure differentially affect both reactions. However this finding does not necessarily mean that there are two different sites in the ribosome implicated in the GTPase activity and might reflect only different binding requirements for the two factors. In fact reconstitution studies of these activities carried out in different laboratories (Ballesta *et al.*, 1971; Hamel *et al.*, 1972; Sander *et al.*, 1972; Ballesta and Vazquez, 1972b; Weissbach *et al.*, 1972) using protein-deficient ribosomal cores from various sources suggest that there are similar structural requirements for both EF T and EF G dependent activities.

The similarity of the EF G dependent GTP hydrolysis and the methanol-induced EF T dependent activity is striking. Both show similar optima for ammonium and magnesium ions and similar K_m for GTP (Nishizuka and Lipmann, 1966). This comparison, however, has to be made with caution due to the presence of methanol in only one system.

The presence of Phe-tRNA at low Mg^{2+} concentrations slightly stimulated EF G dependent GTP hydrolysis probably due to a nonspecific effect (Nishizuka and Lipmann, 1966), but had an inhibitory action at higher Mg^{2+} concentrations. This inhibitory effect has been previously reported and seems to be due to an overlapping of the aminoacyl-tRNA binding site (A site) and the EF G binding site on the ribosome (Modolell and Vazquez, 1973). On the contrary we have not been able to detect inhibition of the EF T dependent reaction by Phe-tRNA under the conditions tested (Figure 4 and Table IV). This might be interpreted *a priori* as an independence of the uncoupled EF T dependent GTP hydrolysis on Phe-tRNA binding to the A site. However it is known that thiostrepton, an antibiotic that possibly binds to the A site (Modolell *et al.*, 1971), does not inhibit but enhances uncoupled EF T dependent GTPase (Ballesta and Vazquez, 1972a); it seems then that the presence of a molecule in the A site does not hinder the interaction of EF T with the ribosome but even facilitates it. In any case it is clear from the results presented in this work that under conditions in which EF G dependent GTP hydrolysis is inhibited by aminoacyl-tRNA binding, uncoupled EF T dependent GTPase is not prevented.

The "unspecific" effect of bulk aminoacylated-tRNA on the uncoupled EF T dependent hydrolysis of GTP (Figure 4 and Table III) is not well understood. Hamel and Nakamoto (1972) have found a similar stimulatory effect by Phe-tRNA at 10 mM Mg^{2+} but unfortunately these authours have not tested other Mg^{2+} concentrations or different kinds of tRNA.

EF T dependent GTP hydrolysis is also uncoupled in the absence of methanol, at high Mg^{2+} concentrations (Figure 4). This uncoupled GTP hydrolysis is also resistant to thiostrepton (Table I). Several authors have detected an uncoupled activity in their systems dealing with EF T. Weissbach *et al.* (1971) have found uncoupled GTP hydrolysis using preisolated aminoacyl-tRNA·EF T_u·GTP complexes to study the binding of Phe-tRNA to the ribosomes. Tetracycline (Ono *et al.*, 1969; Gordon, 1969) or specific ionic conditions (Ravel *et al.*, 1970) also uncouple EF T dependent GTPase. In the absence of mRNA Lin *et al.* (1969) and Hamel *et al.* (1972) also observed an uncoupled GTPase dependent on EF 1 or EF T respectively that was briskly stimulated by aminoacyl-tRNA as in the case of the methanol-induced activity. All these data seem to indicate that methanol is in fact only facilitating a reaction that can also take place in its absence. This observation is highly interesting because it will allow the study of the uncoupled EF T dependent GTPase in the absence of the alcohol, thus making easier the interpretation of the results.

Acknowledgments

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Further Studies on the Properties of Oligonucleotide Cellulose Columns†

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ABSTRACT: Thermal chromatography on columns of deoxyribooligonucleotide cellulose has been used to resolve mixtures of homooligonucleotides differing by one nucleotide residue in length. An extensive examination of the affinity of several series of complementary ribooligonucleotides and deoxyribooligonucleotides for columns of deoxyribooligonucleotide cellulose has confirmed that all the residues in the cellulose-linked oligonucleotide are able to hydrogen bond to their complementary bases. Oligoribouridylates were found to form hybrid structures with deoxyribooligoadenylate cellulose columns having melting temperatures as much as 25° lower than those observed for oligothymidylates of equal length. Comparison of ribooligoadenylates and deoxyribooligoadenylates using columns of oligothymidylate cellulose again showed interactions with ribooligothymidylate to be less stable by 5–10°. A study of the interactions of a series of deoxyribooligonucleotides of mixed repeating base sequence with columns of complementary oligonucleotide cellulose has been made. These results demonstrate that, as expected, the presence of G-C base pairs in the hybrid structure adds considerably to the stability of the hybrid.

The experiments described here extend and amplify the previously reported studies (Astell and Smith, 1972) concerning cellulose-linked deoxyribooligonucleotides of defined length and sequence and the determination of their hydrogen bonding behavior using complementary model oligonucleotides.

Mixtures of deoxyribooligoadenylates have been subjected to thermal chromatography on a column of deoxyribooligothymidylate cellulose and it was found that, in agreement with our earlier conclusions (Astell and Smith, 1972) based on composite elution profiles, consecutive oligomers could be completely resolved.

The affinity of a series of deoxyribooligothymidylates for columns of deoxyribooligoadenylate cellulose has been investigated in a more extensive examination of the number of residues in the cellulose-linked oligonucleotide which are able

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