Interaction of Elongation Factor Tu with the Ribosome. A Study Using the Antibiotic Kirromycin†

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ABSTRACT: Elongation factor Tu (EF-Tu) dependent GTP hydrolysis normally requires the presence of ribosomes and aminoacyl-tRNA (aa-tRNA). In the presence of the antibiotic kirromycin, the factor alone displays a GTPase activity that is enhanced by ribosomes and/or aa-tRNA [Wolf, H., Chinali, G., & Parmeggiani, A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4910–4914]. Using this system, we have found the following: (1) the 50S ribosomal subunit can substitute the 70S ribosome; (2) the 50S CsCl core a, b, and c particles [Sander, G., Marsh, R. C., Voigt, J., & Parmeggiani, A. (1975) *Biochemistry 14*, 1805–1814], lacking an increasing number of proteins, can induce ca. 65, 45, and 25%, respectively, of the EF-Tu·kirromycin GTPase activity of control 50S subunits, in the presence of 30S subunits and aa-tRNA; (3) addition of proteins L7/L12 with L10, but not of proteins L7/L12 free from L10, restored the activity of all the 50S CsCl cores in the EF-Tu·kirromycin-dependent GTPase to 70–90% of the control; (4) proteins L7/L12, with or without contaminating L10, did not induce any EF-Tu-dependent GTPase activity, in contrast to a recent report [Donner, D., Villems, R., Liljas, A., & Kurland, C. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3192–3195], whether EF-Ts and/or kirromycin were present or not.

In the elongation cycle, the interaction between the ternary complex EF-Tu·GTP·aminoacyl-tRNA[1] and the ribosome is associated with the hydrolysis of GTP. Even when uncoupled from polypeptide synthesis this GTPase activity still requires the presence of aminoacyl-tRNA and ribosomes [Gordon, 1969; for reviews, see Lucas-Lenard & Lipmann (1971), Lucas-Lenard & Beres (1974), and Miller & Weissbach (1977)]. The antibiotic kirromycin has proved to be an excellent tool to define precisely the role of the individual components of the EF-Tu-dependent GTPase reaction, since it allows EF-Tu alone to support a turnover GTPase activity which is specifically stimulated by ribosomes or aminoacyl-tRNA or by their combination. By contrast, the complete system including mRNA allows only one round of GTP hydrolysis, since in this condition dissociation of EF-Tu from the ribosome is inhibited by kirromycin (Wolf et al., 1974, 1977). The characterization of the action of the antibiotic has shown that EF-Tu and not the ribosome is the carrier of the catalytic site for the GTP hydrolysis associated with the ternary complex–ribosome interaction. We have used the property of kirromycin to activate the EF-Tu center for GTP hydrolysis as a probe to study the involvement of the ribosomal subunits and their components in this important reaction.

The results presented here show the 50S ribosomal subunit to be far more important than the 30S subunit and that even 50S CsCl cores deprived of a large number of proteins can stimulate the GTPase activity of EF-Tu, and that this activity is further augmented by the ribosomal proteins L7/L12 only after reintegration into the 50S ribosomal particle. The additional presence of aminoacyl-tRNA and of the 30S subunits appears to play a particularly important role for the stimulation by the 50S CsCl cores.

Experimental Procedure

Materials. Electrophoretically homogeneous EF-Tu (Parmeggiani, 1968; Chinali et al., 1977), 0.5 M NH_4Cl-washed ribosomes, ribosomal subunits, 50S CsCl cores a, b, and c (Sander et al., 1975), and Phe-tRNA^{Phe} (45–60% pure; Chinali & Parmeggiani, 1973) were prepared as described. Before zonal centrifugation, ribosomes were preincubated for 20 min at 30 °C at 0.5 mM $MgCl_2$ to give pure subunits as tested by density gradient centrifugation and complementation in polypeptide synthesis. Kirromycin was a gift from Dr. H. Wolf, Tübingen, and was kept as a 10 mM mother solution in 100% ethanol at −30 °C. It was purified as necessary by thin-layer chromatography on silica plates (Merck, Kieselgel 60 F_{254}, 0.5 mm), eluting with a solution of 50 parts chloroform, 47.5 parts methanol, and 1 part concentrated NH_4OH. Pure kirromycin from several sources, as well as the methylated analogue aurodox (X-5108), is revealed as a UV absorbant spot of R_f value 0.7–0.8.

Proteins L7/L12. These were extracted from ribosomes, previously tested to contain very low endogenous GTPase activity, by following the procedure of Hamel et al. (1972). For concentration of the ethanol–NH_4Cl extract, precipitation with acetone at −25 °C was, in two preparations, substituted by concentration in an Amicon apparatus, Model 402, as described by Donner et al. (1978) without apparent advantage. Though the obtained proteins L7/L12 were highly purified, slight amounts of proteins L10 and L25 (<5%) were still present. They were removed by DEAE-Sephadex A-50 chromatography according to Donner et al. (1978), using an NH_4Cl gradient from 0 to 500 mM in 25 mM Tris-HCl, pH 7.5 at 4 °C. This procedure yields pure L7/L12, as judged by electrophoresis and isoelectric focusing with overloaded gels. Amounts of L7/L12 were calculated as the dimeric complex of molecular weight 24 400.

GTPase Assays. The 75-μL reaction mixtures contained the following: 20 mM Tris-HCl (pH 7.8 at 20 °C); 10 mM $MgCl_2$ unless otherwise stated; 10 or 20 pmol of EF-Tu; ribosomes, ribosomal subunits, and 50S-derived particles, each in a twofold excess over EF-Tu; Phe-tRNA^{Phe}, in a fivefold excess over EF-Tu; proteins L7/L12 as indicated; 50 μM kirromycin; 1000 pmol of [γ-^{32}P]GTP (sp act. 200–300 cpm/pmol); 1.3% ethanol carried over with the kirromycin. In the experiments in which the existence of a GTPase activity of

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[1] Abbreviations used: EF-Tu, elongation factor Tu; EF-Ts, elongation factor Ts; EF-G, elongation factor G; Phe-tRNA^{Phe}, tRNA^{Phe} charged to 40–50% with phenylalanine.

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L7/L12 as a function of EF-Tu was examined, we used 10 pmol of dimeric protein L7/L12 and varied the concentration of EF-Tu from 10 to 200 pmol. In these assays, ribosomes, cores, and proteins L7/L12 were preincubated for 30 min at 40 °C in a buffer containing 30 mM Tris-HCl (pH 7.8), 75 mM $MgCl_2$, and 300 mM NH_4Cl prior to adding the remaining components of the GTPase assay. GTPase activity was measured as the amount of $^{32}P_i$ liberated after 10 min of incubation at 30 °C (Sander et al., 1975), for which time the reaction was strictly linear. Radioactivity was measured in an Intertechnique Model SL4000 liquid scintillation spectrometer.

Protein Concentration. The method of Lowry et al. (1951) was used with bovine serum albumin as the standard.

Results

Role of the Ribosomal Subunits in the EF-Tu GTPase Activity. Both ribosomal subunits are needed for the expression of the normal EF-Tu-dependent GTPase activity (Sander et al., 1972, 1975). By contrast, we have also reported that each of the two subunits alone can to a certain extent stimulate the kirromycin-induced GTPase activity, particularly at a large ribosome/EF-Tu ratio (Wolf et al., 1977). We have now studied in more detail these phenomena, paying particular attention to the effect of the monovalent and divalent cations. Recently, we have shown that the GTPase activity of the EF-Tu·kirromycin complex strictly depends on monovalent cations and that this requirement can be overcome by the presence of ribosomes (Sander et al., 1979). We have therefore investigated the Mg^{2+} dependence of the EF-Tu GTPase activity at three concentrations of NH_4^+ (Figure 1). With the simplest assay system, i.e., EF-Tu·kirromycin plus GTP, no GTPase activity could be detected at 2 mM NH_4^+, at all Mg^{2+} concentrations (Figure 1A). Thus, Mg^{2+} cannot replace NH_4^+ in this reaction. As expected, the GTPase activity with the EF-Tu·kirromycin complex rises progressively with increasing [NH_4^+] (compare crosses in panels A, B, and C of Figure 1). However, no dependence on Mg^{2+} was observed between 0.1 and 400 mM.

With both ribosomal subunits present but in the absence of Phe-tRNA^{Phe} (Figure 1, open circles), Mg^{2+} stimulated strongly the GTPase activity, with an optimum near 10 mM between 2 and 200 mM NH_4^+ and an optimum of 40–50 mM at 800 mM NH_4^+. Of the ribosomal subunits, only the 50S subunit showed any significant stimulation (open squares).

This stimulation by the 50S subunit of the EF-Tu·kirromycin GTPase reaction increases greatly in the presence of Phe-tRNA^{Phe} (Figure 1, filled squares), in fact, almost reaching the level of the controls (filled circles) under appropriate conditions. By contrast, the 30S subunit only stimulated the reaction when Phe-tRNA^{Phe} was also present and then only slightly beyond the level reached by Phe-tRNA^{Phe} alone (compare dashed line and filled triangles). We explored this finding by using freshly reactivated 30S subunits (Zamir et al., 1971), with increasing subunit concentrations (not shown), but could not induce any stimulation of the EF-Tu·kirromycin GTPase except in the presence of Phe-tRNA^{Phe}, and maximum stimulation was attained with an excess of between 1.5- and 2-fold 30S subunits over EF-Tu, with or without the reactivation treatment. This demonstrates that the 50S subunit has a much greater impact on the EF-Tu GTPase reaction than the 30S subunit. As in the absence of Phe-tRNA^{Phe}, also in its presence there is a strong stimulation by Mg^{2+}. Increasing NH_4^+ concentration again shifted the Mg^{2+} optimum toward higher values, particularly with the 50S subunit. Such an interdependence has been shown to exist also

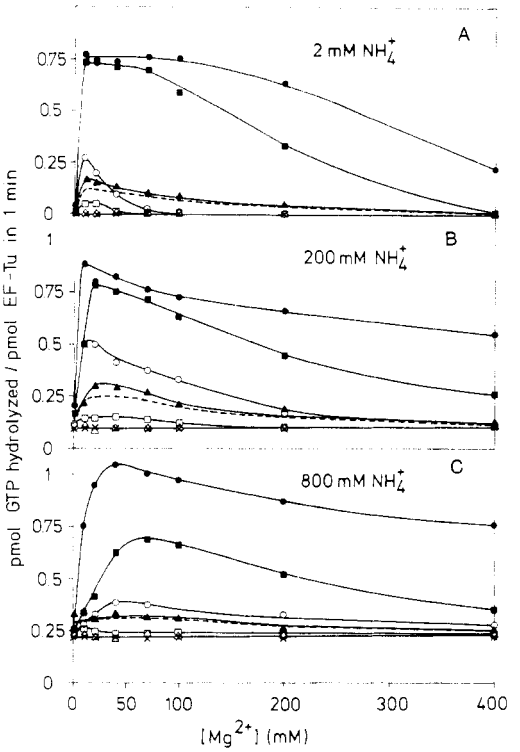

FIGURE 1: [Mg^{2+}] dependence of kirromycin-induced EF-Tu GTPase at various NH_4^+ concentrations. Role of ribosomal subunits and Phe-tRNA^{Phe}. The 75-μL reaction mixtures contained 20 pmol of EF-Tu, 40 pmol of 50S and/or 30S subunits and 100 pmol of Phe-tRNA^{Phe} as indicated, 50 μM kirromycin, and 1 nmol of [γ-^{32}P]GTP. (×) EF-Tu; (△) EF-Tu plus 30S subunits; (□) EF-Tu plus 50S subunits; (○) EF-Tu plus 50S and 30S subunits. Filled symbols: the same in the presence of Phe-tRNA^{Phe}. Dashed line: activity with EF-Tu and Phe-tRNA^{Phe} in the absence of ribosomes. After a 10-min incubation, GTP hydrolysis was measured as described under Experimental Procedure.

for the EF-G-dependent GTPase activity (Parmeggiani et al., 1974) and poly(U)-dependent poly(phenylalanine) synthesis (Bogatyreva et al., 1970).

Components of the 50S Subunit Implicated in EF-Tu-Dependent GTPase Activity. Action of 50S CsCl Cores. We have previously described a series of protein-deficient 50S subparticles prepared by isopycnic centrifugation in 5 M CsCl, named 50S CsCl cores a, b, and c (Sander et al., 1975), whose protein composition is shown in Table I. These cores contained no proteins L7, L12, or L10. All of them contain normal amounts of 5S RNA and are able to associate with 30S subunits (Sander et al., 1975; Sander & Parmeggiani, 1976; Sander, 1977). Their stimulation of the EF-Tu·kirromycin GTPase reaction in the presence of 30S subunits and Phe-tRNA^{Phe} is shown in Figure 2. At sufficiently high Mg^{2+}, the presence of each of these cores increased the GTPase activity beyond that found with EF-Tu·kirromycin plus only 30S subunits and Phe-tRNA^{Phe}; core a was most active followed by cores b and c. This behavior resembles the EF-T GTPase activities obtained previously with the same CsCl cores in the presence of excess L7/L12 (Sander et al., 1975). It should be noted that proteins L7/L12 were neither added in the experiments of Figure 2 nor present in the cores, as judged by two-dimensional electrophoresis with overloaded gels.

Thus, in the presence of kirromycin, L7/L12 are no longer essential to stimulate GTPase activity in the presence of 30S subunits, 50S CsCl cores and Phe-tRNA^{Phe}.

The kinetics of the EF-Tu GTPase activity stimulated by the 50S CsCl cores at 50 mM Mg^{2+} and 200 mM NH_4^+ is shown in Figure 3. The reaction was linear up to the 10-min

Table I: Protein Composition of Various 50S CsCl Cores[a]

protein	CsCl core a	CsCl core b	CsCl core c
L1	–	–	–
L2	+	+	+
L3	+	+	+
L4	+	+	+
L5	+	+	–
L6	+	–	–
L7	–	–	–
L8	–	–	–
L9	+	+	(+)
L10	–	–	–
L11	+	+	+
L12	–	–	–
L13	+	+	+
L14	+	+	(+)
L15	+	+	–
L16	–	–	–
L17	+	+	+
L18	+	+	–
L19	+	+	(+)
L20	+	+	+
L21	+	+	+
L22	+	+	+
L23	+	+	+
L24	+	+	+
L25	–	–	–
L27	+	+	–
L28	+	+	–
L29	+	+	+
L30	+	+	–
L32	+	+	+
L33	–	+	–

[a] Protein content was determined by using two-dimensional polyacrylamide gel electrophoresis (Kaltschmidt & Wittmann, 1970). Symbols: +, present in normal amount (70–100% of the control); (+), present in reduced amount (<50%); –, not present. Neither L26 nor L31 was observed in any case. The results represent the average of five different preparations.

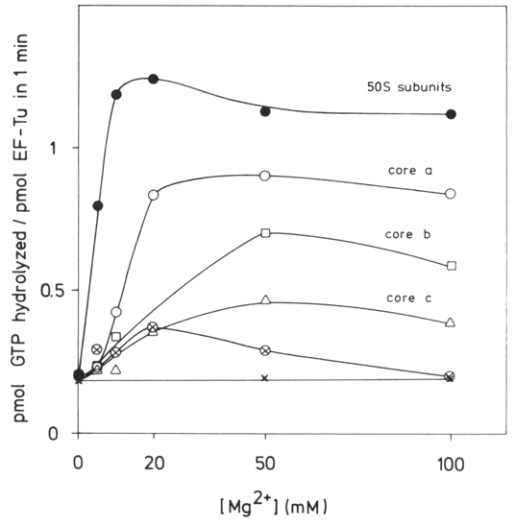

FIGURE 2: Stimulation by various 50S CsCl cores of the kirromycin-induced EF-Tu GTPase activity at 200 mM NH_4^+ as a function of [Mg^{2+}]. The reaction mixtures contained 10 pmol of EF-Tu, 20 pmol of 50S subunits or CsCl cores, 30 pmol of 30S subunits, and 50 pmol of Phe-tRNA^{Phe} as indicated, 50 μM kirromycin, and 1 nmol of [γ-^{32}P]GTP. (×) EF-Tu alone; all other assays with Phe-tRNA^{Phe} plus (⊗) 30S subunits, (●) 50S subunits, (○) 50S CsCl core a and 30S subunits, (□) 50S CsCl core b and 30S subunits, and (△) 50S CsCl core c and 30S subunits.

incubation time chosen for all of the other experiments. The figure also demonstrates that, in contrast to the 50S subunit, stimulation by the 50S CsCl cores in the presence of Phe-tRNA^{Phe} depends strongly on the 30S subunit (compare panels

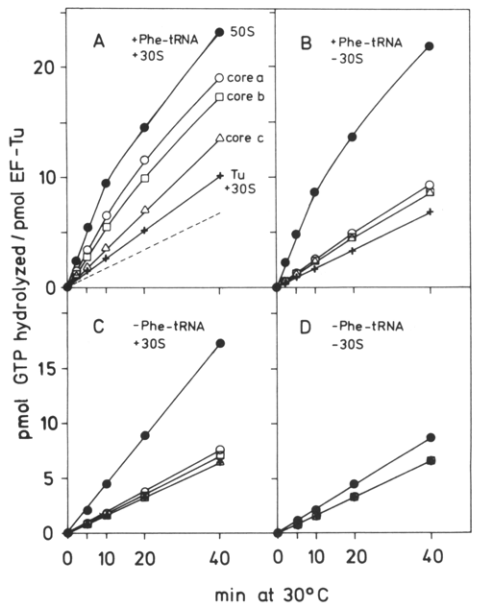

FIGURE 3: Kinetics of EF-Tu·kirromycin GTPase activity stimulated by 50S CsCl cores at 200 mM NH_4^+ and 50 mM Mg^{2+}. Reaction mixtures contained 10 pmol of EF-Tu, 1.4 nmol of [γ-^{32}P]GTP, 50 μM kirromycin, and 20 pmol of 50S particles, 20 pmol of 30S subunits, and 50 pmol of Phe-tRNA^{Phe} as indicated. All 50S particles were preincubated for 30 min at 30 °C in 20 mM Tris-HCl (pH 7.8) containing 300 mM NH_4^+ and 10 mM Mg^{2+}. (+) EF-Tu; (●) EF-Tu plus 50S subunits; (○) EF-Tu plus 50S CsCl core a; (□) EF-Tu plus 50S CsCl core b; (△) EF-Tu plus 50S CsCl core c. Additions: (A) 30S subunits and Phe-tRNA^{Phe}; (B) Phe-tRNA^{Phe}; (C) 30S subunits; (D) none. Dashed line: EF-Tu·kirromycin in the absence of ribosomal particles.

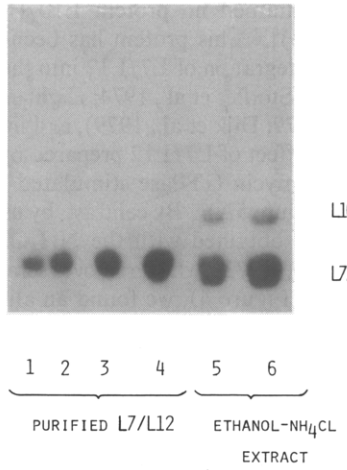

FIGURE 4: Slab gel electrophoresis in 18% acrylamide and 0.1% sodium dodecyl sulfate of purified proteins L7/L12 (slots 1–4) and of ribosomal ethanol–NH_4Cl extracts (slots 5 and 6). Buffers were essentially those of Davis (1964); the concentrating gel was 4% acrylamide. Electrophoresis was carried out for 5 h at 75 V in a Savant slab gel apparatus, and the gels were stained with 0.1% Coomassie brilliant blue in 50% methanol–7.5% acetic acid and destained by shaking in 5% methanol–10% acetic acid. Slots 1–4: 10.8, 21.2, 43.2, and 81 μg of L7/L12 purified according to Donner et al. (1978). Slots 5 and 6: 32 and 48 μg, respectively, of the ethanol–NH_4Cl extract (Hamel et al., 1972). Two-dimensional electrophoresis according to Kaltschmidt & Wittmann (1970) confirmed the identity of the contaminating spot as protein L10.

A and B). Without Phe-tRNA^{Phe}, stimulation of the EF-Tu·kirromycin GTPase activity by the cores was very low in the presence of 30S subunits and zero in their absence (panels C and D).

Reconstitution of GTPase Activity to 50S CsCl Cores by Proteins L7/L12. Proteins L7/L12 purified on DEAE-

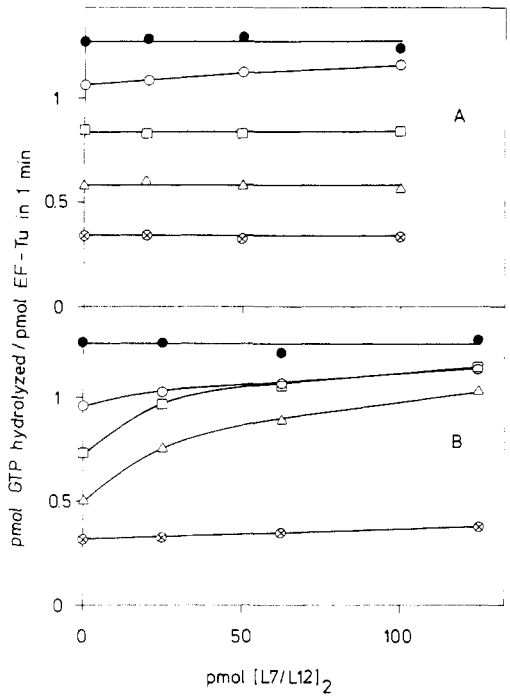

FIGURE 5: Complementation of 50S CsCl cores with pure L7/L12 (A) or L7/L12 and L10 (B) at 200 mM NH_4^+ and 50 mM Mg^{2+}. The reaction mixtures contained 10 pmol of EF-Tu, 30 pmol of 30S subunits with or without 20 pmol of 50S subunits or 50S CsCl cores, 50 pmol of Phe-tRNA^{Phe}, 1 nmol of [γ-^{32}P]GTP, and 50 μM kirromycin, plus the indicated amounts of proteins L7/L12. (⊗) 30S subunits; (●) 50S plus 30S subunits; (○) 50S CsCl core a, (□) 50S CsCl core b, and (△) 50S CsCl core c, all with 30S subunits.

Sephadex A-50 contained no protein L10 [Figure 4 and Donner et al. (1978)]. This protein has been described as essential for the reintegration of L7/L12 into the 50S particle (Schrier et al., 1973; Stöffler et al., 1974; Highland & Howard, 1975; Pettersson, 1979; Dijk et al., 1979), and indeed we were not able to see any effect of L7/L12 prepared by this method on the EF-Tu·kirromycin GTPase stimulated by 50S CsCl cores or subunits (Figure 5A). By contrast, by using an excess of proteins L7/L12 obtained with the NH_4Cl–ethanol extraction (Hamel et al., 1972), which removes some protein L10 from the ribosome (Figure 4), we found an almost total reconstitution of the EF-Tu·kirromycin GTPase activity with all the 50S CsCl cores (Figure 5B). We have moreover repeated our previous experiments (Sander et al., 1975) and found that, also without kirromycin, reconstitution of the EF-Tu GTPase activity to the reported levels (core a, 80%; core b, 50%; core c, 20% of the activity with the 50S subunit at 30 mM $MgCl_2$) is possible only with the preparation containing contaminating protein L10. Apparently our proteins L7/L12 utilized for the experiments reported by Sander et al. (1975) contained some protein L10.

The importance of protein L10 for reintegrating proteins L7/L12 into the ribosome (Schrier et al., 1973; Stöffler et al., 1974; Highland & Howard, 1975; Pettersson, 1979; Dijk et al., 1979) was also evident when we used the NH_4Cl–ethanol-extracted 50S particles, which contain protein L10 (Highland & Howard, 1975; Ballesta & Vazquez, 1974). This particle could be completely reactivated by proteins L7/L12 purified by the method of Donner et al. (1978), stimulating both the EF-Tu- and EF-G-dependent GTPase activities (data not shown).

Effect of Proteins L7/L12 on the EF-Tu GTPase Activity in the Absence of Ribosomal Particles. In a recent publication Kurland and co-workers have reported that purified L7/L12

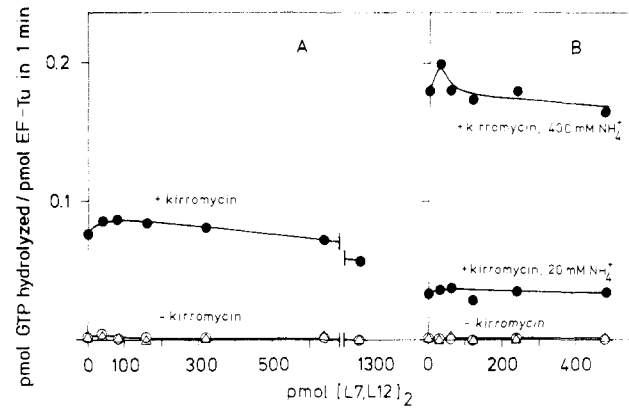

FIGURE 6: Proteins L7/L12 do not induce EF-Tu GTPase activity. (A) The 75-μL reaction mixtures contained, in 20 mM Tris-HCl (pH 7.5), 30 mM KCl, 30 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, and 150 pmol of [γ-^{32}P]GTP (sp act. 2580 cpm/pmol), (○) 50 pmol of EF-Tu, (△) 50 pmol of EF-Tu plus 500 pmol of EF-Ts, and (●) 50 pmol of EF-Tu plus 50 μM kirromycin. (B) The 75-μL reaction mixtures contained, in 30 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, the indicated NH_4Cl concentration, 1 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, and 150 pmol of [γ-^{32}P]GTP (sp act. 1400 cpm/pmol), (○) 20 pmol of EF-Tu, (△) 20 pmol of EF-Tu plus 20 pmol of EF-Ts, and (●) 20 pmol of EF-Tu and 50 μM kirromycin. The blanks obtained with proteins L7/L12 alone (maximally 0.01 pmol of GTP hydrolyzed per min per tube with the highest amount of L7/L12) have been subtracted.

are able to induce the EF-Tu GTPase reaction in the absence of ribosomes (Donner et al., 1978). This activity was stimulated up to 10 times by the addition of EF-Ts. We have carefully repeated these experiments (Figure 6A) and found no effect by L7/L12 over the whole concentration range examined, whereas these authors have reported values of 0.4 pmol of GTP hydrolyzed per pmol of EF-Tu in 1 min. Neither have we found any activity in the additional presence of 30S subunits and/or aa-tRNA. An important difference between our system and that of Kurland and co-workers is their high GTPase activity already obtained with EF-Tu plus EF-Ts in the absence of L7/L12 (0.1 pmol of GTP hydrolyzed per pmol of EF-Tu in 1 min under the same conditions as used in Figure 6A). In the presence of kirromycin (filled symbols, Figure 6), proteins L7/L12 at approximately equimolar concentration to EF-Tu marginally stimulated the GTPase activity of EF-Tu, whereas they slightly inhibited the reaction when present in a large excess. This is particularly interesting since the ionic conditions of the experiment described in Figure 6A are the same as those used by Kurland and co-workers.

In a further series of experiments, we were also unable to detect any induction of GTPase activity, when (I) L7/L12 were added in great excess to EF-Tu alone over a large range of monovalent cation concentrations (Figure 6B) and (II) increasing concentrations of EF-Tu (up to 20 times) were added to 10 pmol of dimeric L7/L12 (not shown). We consider this experiment critical for denying the existence of a catalytic site for GTP hydrolysis located on L7/L12, as suggested by the experiments of Kurland and co-workers (Donner et al., 1978).

We conclude from our results that isolated proteins L7/L12 are unable to induce the GTPase activity of EF-Tu in the presence or absence of EF-Ts. The same results were found when we used the L7/L12 preparation containing some protein L10 (not shown).

Discussion

The EF-Tu-dependent GTPase reaction can be envisaged as a process centered around the elongation factor that itself

carries the catalytic site (Chinali et al., 1977) and is allosterically controlled by its various effectors. The existence of independent binding sites on EF-Tu for GTP/GDP and aa-tRNA, enabling the formation of the ternary complex EF-Tu·GTP·aa-tRNA, is well established [for reviews, see Lucas-Lenard & Beres (1974) and Miller & Weissbach (1977)]. The case of elongation factor Ts is less clear, since it competes for binding to EF-Tu with both GTP–GDP and kirromycin, which in turn have independent binding sites on EF-Tu (Wolf et al., 1974; Chinali et al., 1977). It is, however, not known whether these phenomena are a consequence of partially overlapping sites for GTP/GDP, kirromycin, and EF-Ts or rather the result of long-range effects which lead to allosteric interference between different sites.

Thanks to the action of kirromycin, which in the absence of mRNA induces a turnover GTPase activity (Wolf et al., 1977), we have been able to define more clearly the interacting regions of the EF-Tu molecule and the ribosome. Our results suggest that this region is composed of several sites. There would appear to be at most a weak interaction with the 30S ribosomal subunit, which is in evidence only when the subunits are in excess over EF-Tu and in the presence of aa-tRNA.

The interaction with the 50S subunit is much stronger and is independent of, although stimulated by, aa-tRNA (see Figures 1 and 3). That the GTPase activity with kirromycin is reduced progressively, but not abolished completely, on passing from 50S subunits to cores a, b, and c implies a complex interaction between EF-Tu and the subunit, involving several different proteins. The pivotal role of protein L6 (the only one lost upon going from core a to core b) is again evident (Schrier et al., 1973; Sander et al., 1975; Sander & Parmeggiani, 1976) together with the relative unimportance of proteins L1, L10, L16, L25, and L33, which are missing from CsCl core a, in addition to proteins L7/L12. It should be emphasized that the relative activities of the cores a, b, and c, in the absence of proteins L7/L12 but with kirromycin, follow the same order as those found previously with L7/L12 in the absence of the antibiotic (Sander et al., 1975) and suggest that in this system proteins L7/L12 can only be acting in an auxiliary role. These results confirm that protein L10 is essential for the reintegration of proteins L7/L12 into the 50S particles and is not directly implicated in the EF-Tu GTPase reaction (Schrier et al., 1973; Stöffler et al., 1974; Highland & Howard, 1975; Pettersson, 1979; Dijk et al., 1979).

Proteins L7/L12 present curious properties. On the one hand, they are apparently the most important ribosomal proteins implicated in the physiological EF-Tu GTPase reaction [Hamel et al., 1972; Sander et al., 1972, 1975; see also the review by Möller (1974)], though this property pertains only when they are an integral part of the 50S ribosomal subunit. In properly chosen conditions, on the other hand, in the absence of these proteins not only does the EF-Tu GTPase reaction function well [Sander et al. (1975) and this work] but also polypeptide synthesis in vitro has been reported (Glick, 1977; Koteliansky et al., 1977). At present, there appears no way to explain the considerable discrepancy between our results and those of the group of Kurland, following our failure to observe any effect on EF-Tu GTPase by the proteins L7/L12 separated from the 50S particle. In this regard, we wish to stress that these experiments have been repeated a large number of times with different preparations.

The CsCl core c contains only 13 major proteins (L2, L3, L4, L11, L13, L17, L20, L21, L22, L23, L24, L29, and L32), the ribosomal RNA thus forming over 80% of the particle. Yet EF-Tu·kirromycin GTPase activity is stimulated considerably by its presence even in the absence of proteins L7/L12 and L10, raising the possibility of a direct participation of 23S RNA and/or 5S RNA in this reaction. However, this seems unlikely since with intact particles there is no interdependence between the optima for monovalent cations and pH (Sander & Crechet, 1978), as might be expected in an enzymatic system having RNA in the neighborhood of the catalytic site (Douzou & Maurel, 1977; Sander et al., 1978; Beaudry et al., 1979). Moreover, 16S, 23S, and 5S RNA, when tested in this system, gave no stimulation of the GTPase activity (data not shown).

In the system containing kirromycin, the other EF-Tu effector, aminoacyl-tRNA, appears to contribute independently toward the conformation of the factor leading to GTP hydrolysis, such that, when acting in concert, ribosomes and aa-tRNA stimulate more than the sum of each acting individually and are always required together for maximum GTPase activity.

Taken together, these results demonstrate the synergistic functioning of a very complex ribosomal system, which is responsible for triggering the GTPase activity of EF-Tu, and deny a simplistic ribosome–EF-Tu interaction, as has been suggested (Pongs et al., 1974; Pongs, 1978; Donner et al., 1978).

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Binding of a Tryptophan-Containing Peptide (Lysyltryptophyllysine) to Deoxyribonucleic Acid Modified by 2-(*N*-Acetoxyacetylamino)fluorene†

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ABSTRACT: The binding of the tripeptide Lys-Trp-Lys to DNA modified by reaction with the chemical carcinogen 2-(*N*-acetoxyacetylamino)fluorene (AAAF) has been investigated by fluorescence spectroscopy. A quenching of tryptophan fluorescence was observed which increased when the degree of base substitution by AAAF increased. Similar results were obtained with the 7-iodo derivative of AAAF (AAAIF). Two hypotheses are discussed which could account for the experimental results: (1) stacking interactions of the tryptophyl residue of the peptide with nucleic acid bases in locally unpaired regions in the vicinity of modified bases; (2) energy transfer from the tryptophyl residue of the peptide to acetylaminofluorene bound to guanine bases without direct interaction of this residue with nucleic acid bases (outside binding). The results obtained with denatured DNA in the absence and the presence of chemical modifications by AAAF or AAAIF allow us to conclude that energy transfer contributes to fluorescence quenching in the case of AAIF but not in that of AAF. Stacking interactions are therefore responsible for fluorescence quenching of Lys-Trp-Lys when bound to AAF-modified DNA. In the case of DNA-AAIF, fluorescence quenching is due both to energy transfer and to stacking of the tryptophan ring with bases inside the helix. These results are discussed in relation to what is already known in terms of local structure and with respect to the role that could be played by aromatic residues of proteins in the recognition of chemically damaged DNA.

The binding of peptides containing tryptophan and basic residues (such as Lys-Trp-Lys) to nucleic acids has been shown to involve two types of complexes according to the scheme (Brun et al., 1975; Toulmé & Hélène, 1977; Maurizot et al., 1978)

$$\text{nucleic acid} + \text{peptide} \underset{K_1}{\rightleftharpoons} \text{complex I} \underset{K_2}{\rightleftharpoons} \text{complex II} \quad (1)$$

Electrostatic interactions between lysyl side chains and phosphate groups are involved in both complexes. In complex I the aromatic amino acid side chain does not interact with nucleic bases while stacking interactions take place in complex II (Dimicoli & Hélène, 1974a,b). The formation of complex II is strongly favored if the nucleic acid is single stranded (K_2 is much higher for single strands than for double strands) (Toulmé et al., 1974; Toulmé & Hélène, 1977).

If native DNA is submitted to UV irradiation, several damages occur, among which pyrimidine dimers have the more important biological role. The DNA region around such defects has an altered conformation. Hydrogen bonds are disrupted, leading to locally opened structures. It was previously shown that the tryptophyl residue of Lys-Trp-Lys was preferentially stacked with bases in these single-stranded regions (Toulmé et al., 1974; Toulmé & Hélène, 1977).

Chemical reaction of DNA bases with metabolites of strong carcinogenic compounds also leads to altered regions in the DNA double helix [for a review see Daune & Fuchs (1977)].

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