Ribosomal Protein L1 from Escherichia coli

ITS ROLE IN THE BINDING OF tRNA TO THE RIBOSOME AND IN ELONGATION FACTOR G-DEPENDENT GTP HYDROLYSIS

(Received for publication, April 6, 1982)

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Two Escherichia coli mutants lacking ribosomal protein L1, previously shown to display 40 to 60% reduced capacity for in vitro protein synthesis (Subramanian, A. R., and Dabbs, E. R. (1980) Eur. J. Biochem. 112, 425–430), have been used to study partial reactions of protein biosynthesis. Both the binding of N-acetyl-Phe-tRNA to ribosomes and the 6 to 8-fold stimulation of the elongation factor G (EF-G)-dependent GTPase reaction by mRNA plus tRNA, assayed in the presence of wild type 30 S subunits, were low with L1-deficient 50 S subunits. Addition of pure protein L1 to the assay restored both reactions to 100% of the control. By contrast, the basic EF-G GTPase reaction in the absence of mRNA and tRNA was not at all affected (mRNA alone had no effect). None of the following partial reactions were more than moderately modified by the lack of protein L1: binding to ribosomes of EF-G GDP plus fusidic acid; the translocation reaction catalyzed by EF-G plus GTP; poly(U)-dependent binding to ribosomes of Phe-tRNA\(^{\text{Phe}}\) (whether dependent on elongation factor Tu plus GTP or not); and the EF-Tu-dependent GTPase activity.

It is concluded that protein L1 is involved in the interaction between ribosomes and peptidyl-tRNA (or tRNA) in the peptidyl site and consequently in the ribosomal GTPase activity depending on the simultaneous action of tRNA and EF-G.

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The basic functions of the ribosome during protein biosynthesis are known (see Refs. 1 and 2 for review). However, the roles of many of the individual ribosomal components in this process are still unclear. Of the 53 Escherichia coli ribosomal proteins, only relatively few have been assigned clear functions. Thus, e.g. protein L7/L12 of the large ribosomal subunit is important for elongation- and initiation factor-dependent ribosomal reactions (3, 4), and protein L16 is pivotal for peptidyltransferase activity (5) and protein S1 for mRNA binding to ribosomes (6–8). Frequently, however, the large number of ribosomal proteins seemingly implicated makes it difficult to evaluate the contribution of individual ribosomal proteins to a given function (see Ref. 1). Recently, E. coli mutants lacking one protein at a time have become available (9, 10), which should prove useful here.

Two independently isolated mutants of E. coli, both lacking protein L1 from the large ribosomal subunit and designated RD19 and MV17-10, respectively, have been described (9, 10). Subramanian and Dabbs (11) analyzed ribosomes from these strains in vitro for protein synthesis using poly(U) and phage f2 RNA as messengers and found them to be 40 to 60% as active as wild type ribosomes; full activity could be recovered by adding pure protein L1. Here we report on the analysis of partial reactions of polypeptide synthesis using these L1-deficient 50 S subunits.

MATERIALS AND METHODS

Assays.—Unless otherwise stated, assays were carried out at 30 °C in 50-μl reaction mixtures containing 50 mM imidazole acetate (pH 7.5), 100 mM KCl, 20 μmol of control 30 S subunits (heat-activated by a 30-min incubation at 40 °C), and 3 to 20 pmol of 50 S subunits. In most of the assays shown in the figures, equimolar amounts of 30 and 50 S subunits were used; however, all assays were also performed using limiting amounts of 30 S subunits and, where possible, conditions of linear kinetics. In some cases (e.g. enzymatic binding of Phe-tRNA to poly(U)-ribosomes), the mutant 50 S subunits had even higher activities than the controls under these conditions, while in most assays there was no difference in the results reported.

Binding of EF-G to ribosomes was carried out in 40 mM imidazole acetate, 10 mM MgCl₂, 20 or 100 mM KCl, 0.5 mM fusidic acid, 4.2 μM (210 pmol) [γ-32P]GTP (280 cpm/pmol), 40-μl aliquots were pipetted into 2 ml of cold wash buffer (10 mM imidazole acetate, 10 mM MgCl₂, 0.02 mM fusidic acid) previously placed on top of nitrocellulose filters (Sartorius SM 11306), followed by immediate suction and washing with 2 ml of wash buffer. The dried filters were counted in toluene containing 2 g/liter of 2,5-diphenyloxazole.

Translocation of N-Acetyl-Phe-tRNA from the Ribosomal A Site to the P Site—For the binding of tRNA\(^{\text{Phe}}\) to the P site, the 30-μl reaction mixtures (25 mM imidazole acetate, 5 mM MgCl₂, 160 mM NH₄Cl) contained 15 pmol of 30 S subunits, 2 to 15 pmol of 50 S subunits, 8 μg of poly(U), and 11 pmol of uncharged tRNA\(^{\text{Phe}}\).

Incubation was for 10 min. For the binding of N-acetyl-Phe-tRNA to the A site (at 0 °C), the MgCl₂ concentration was raised to 20 mM and 16 pmol of N-acetyl-[14C]Phe-tRNA\(^{\text{Phe}}\) were added. For translocation, immediately following the second step, the indicated amounts of EF-G plus 2.5 to 20 pmol of GTP were added and the reaction mixtures were incubated for 10 min at 30 °C, then cooled to 0 °C. For puromycin reaction, puromycin was added to 1 mM final concentration followed by a 30-min incubation at 30 °C. By this time, all salt and buffer concentrations were reduced by one fifth compared with the first step except [Mg\(^{2+}\)] which was 15 mM. After cooling to 0 °C, 200 μl of 1 M NH₄HCO₃, and 1 ml of ethyl acetate were added;

1 Portions of this paper (including part of "Materials and Methods" and additional Figs. 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9656 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-905, cite the author, and include a check or money order for $1.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviations used are: EF, elongation factor; A site, aminoacyl site; P site, peptidyl site.
following vigorous shaking for 30 s, an aliquot of the organic supernatant was counted in toluene/Triton X-100 (Serva) containing 10 g/liter of 2,5-diphenyloxazole and 0.1 g/liter of 1,4-bis[2-(5-phenyloxazolyl)]benzene.

The EF-G GTPase reaction was carried out for 10 min in 100-μl reaction mixtures (160 mM KCl, MgCl₂ as indicated) with 100 pmol of EF-G and 20 to 30 pmol of [γ-32P]GTP (5 to 10 cpm/pmol). 10 pmol of tRNA, or N-acetyl-Phe-tRNA and 6 μg of poly(A), 100 pmol of EF-Tu, 20 pmol of EF-Ts (this EF-Tu/EF-Ts ratio being optimal in our conditions), and 80 to 300 pmol of [γ-32P]GTP (1000 to 2500 cpm/pmol). The samples were processed as for the EF-G GTPase reaction. In both kinds of GTPase reaction, the highest activities never exceeded 20% hydrolysis of the total substrate.

Binding of aminoacyl-tRNA to ribosomes was carried out essentially as described (21) using 20 pmol of [14C]Phe-tRNA, N-acetyl-[14C]Phe-tRNA, or [14C]Lys-tRNA, plus 40 pmol of EF-Tu and 400 pmol of GTP where indicated.

RESULTS

The two-dimensional gel electrophoreses (22) of the proteins contained in 50 S subunits from E. coli wild type and mutant strains are shown in Fig. 1. Only the wild type contained the full complement of proteins, while 50 S subunits from both mutant strains lacked protein L1.

The individual reactions of polypeptide synthesis have been studied in the order in which they take place on the ribosome, starting with the binding of aminoacyl-tRNA to ribosomes.

Binding of Aminoacyl-tRNA to Ribosomes—Fig. 2 shows the poly(U)-directed binding of Phe-tRNA to ribosomes as a function of [Mg²⁺]. The EF-Tu- and GTP-dependent or “enzymatic” binding (upper curves) was nearly the same for all of the strains. In the 3 to 15 mM range of [Mg²⁺], nonenzymatic binding was (reproducibly) slightly higher with the mutants than with the control 50 S subunits, but this effect disappeared with short incubation times (1 min) and was reversed when Lys-tRNA plus poly(A) replaced Phe-tRNA and poly(U) (data not shown). Moreover, with rate-limiting amounts of 50 S subunits and 16-s incubations at 0 °C, the mutant 50 S subunits showed slightly higher stimulation of the enzymatic binding and lower nonenzymatic binding of Phe-tRNA than the controls (not shown). Therefore, binding of aminoacyl-tRNA to the A site (whether EF-Tu- and GTP-dependent or not; Refs. 23–25) is not influenced by the lack of protein L1.

N-Acetyl-Phe-tRNA is preferentially bound to the P site (Ref. 26; checked with the puromycin reaction). This reaction was studied at 7 and 15 mM Mg²⁺ by adding increased amounts of the various 50 S subunits to control 30 S subunits (Fig. 3). The binding was very fast, and the kinetics could not be measured by our technique. A 30-s incubation was nonetheless chosen, at which point binding tapers off but still increases with time.

Lack of protein L1 strongly reduced N-acetyl-Phe-tRNA binding at 7 mM Mg²⁺ (Fig. 3A) and totally abolished it at 15 mM Mg²⁺ (Fig. 3B). Adding back L1 restored this activity in both cases.

EF-Tu-dependent GTPase Activity—This reaction normally follows the enzymatic binding of aminoacyl-tRNA and precedes release of the factor. It normally requires both ribosomal subunits and aminoacyl-tRNA, whereas cognate mRNA is needed only at low (4 to 10 mM) Mg²⁺ concentration (21). The activity was measured as a function of aminoacyl-tRNA concentration at 6 and 30 mM Mg²⁺ (see Miniprint); in no case was there a significant effect when 50 S subunits lacking protein L1 replaced control subunits.

Binding of EF-G to Ribosomes—In the presence of the antibiotic fusidic acid, EF-G-GTP or EF-G-GDP bind to both 50 S subunits and 70 S ribosomes (27, 28), which in turn bind to nitrocellulose filters. We studied this reaction using wild type and mutant 50 S subunits (see Miniprint) and found no significant difference. Therefore, the binding of EF-G to ribosomes does not require protein L1.

The Translocation Reaction and Peptidyltransferase—To study this reaction, the next in the sequence leading to the addition of an amino acid to the polypeptide chain, we made use of an artificial system involving a number of steps (see “Materials and Methods”); no dependence on the presence of protein L1 was found (see Miniprint).

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FIG. 2. Binding of tRNA to ribosomes. Wild type 30 S subunits and the indicated 50 S subunit species were preincubated for 10 min at 5 mM Mg$^{2+}$ with poly(U) and uncharged tRNA$^{\text{Phe}}$ to occupy the P site and then reacted for 10 min at 30 °C with either [3H]Phe-tRNA$^{\text{Phe}}$ (filled symbols) or preformed (10 min, 30 °C) ternary complex EF-Tu-GTP-[3H]Phe-tRNA (open symbols). O, control; △, MV17-10 50 S subunits; ■, RD19 50 S subunits.

The EF-G GTPase Reaction in the Presence of mRNA and tRNA—mRNA and tRNA have been reported both to stimulate (30, 31) and to inhibit (32, 33) the EF-G GTPase reaction, the latter most strongly at high [Mg$^{2+}$]. As Parmeggiani et al. (34) and Chinali and Parmeggiani (35) have shown, the stimulation by tRNA of the EF-G GTPase is strongly enhanced at high concentrations of monovalent cations and low (5 to 10 mM) Mg$^{2+}$ concentrations. We have confirmed this using isolated 30 and 50 S subunits. The conditions chosen for most of our experiments (160 mM K$^+$, 7 mM Mg$^{2+}$) maximize the stimulation of the EF-G GTPase reaction by tRNA$^{\text{Phe}}$ and poly(U). Fig. 4A shows the effect of poly(U) and tRNA$^{\text{Phe}}$ on the EF-G GTPase reaction at 160 mM K$^+$. Their addition results both in a strong stimulation of the GTPase activity at moderate Mg$^{2+}$ concentrations and a shift of the Mg$^{2+}$ optimum toward lower concentrations. The addition of poly(U) alone showed neither of these effects (data not shown). Fig. 4B shows the same assay performed with 50 S subunits from mutant MV17-10 (similar results were obtained with RD19) supplemented with wild type 30 S subunits. The Mg$^{2+}$ dependence of the basic GTPase reaction was similar to the control, while the stimulation by tRNA$^{\text{Phe}}$ and poly(U) was lower for the mutant 50 S subunits, particularly between 6 and 8 mM Mg$^{2+}$. This pointed to a possible role of protein L1 in the interaction among ribosomes, tRNA, and EF-G.

Fig. 5 shows the effect on GTP hydrolysis of adding purified protein L1 without and with poly(U) and tRNA$^{\text{Phe}}$. In the absence of tRNA$^{\text{Phe}}$ and poly(U), protein L1 had no significant effect on GTPase activity with mutant and control 50 S subunits. The stimulation of the EF-G GTPase by tRNA$^{\text{Phe}}$ and poly(U) was fully dependent on protein L1 at 7 mM Mg$^{2+}$, while at 10 mM Mg$^{2+}$ the importance of L1 for the tRNA effect was less pronounced, 50 S subunits from mutant MV17-10 displaying 50% of the full effect in the absence of L1. The protein did, however, restore activity to 100% of the control with both MV17-10 and RD19 50 S subunits.

The effect of increasing amounts of tRNA$^{\text{Phe}}$ at 7 mM Mg$^{2+}$ is shown in Fig. 6. Maximum stimulation was achieved at [tRNA]/[ribosomes] ~ 0.5. At this low stoichiometry and in the ionic conditions employed, uncharged tRNA binds almost exclusively to the ribosomal P site (23-26,36). N-Acetyl-Phe-tRNA$^{\text{Phe}}$ bound to the P site (checked by the puromycin reaction) also stimulated the EF-G GTPase reaction in the presence of protein L1.

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FIG. 5. Effect of protein L1 on the stimulation by tRNA\textsuperscript{Phe} and poly(U) of the EF-G GTPase reaction at 7 and 10 mM Mg\textsubscript{2+}. Control 50S (O), MV17-10 50S (C), and RD19 50S (△) subunits, all complemented with wild type 30S subunits. The 50S subunits were preincubated for 10 min at 40 °C with the indicated amounts of protein L1.

FIG. 6. Effect of tRNA on the EF-G GTPase reaction in the absence and presence of protein L1 at 7 mM Mg\textsubscript{2+}. Control 50S (O), MV17-10 50S (C), and RD19 50S (△) subunits, all with wild type 30S subunits. Where indicated, the 50S subunits were incubated for 10 min at 40 °C with 80 pmol of protein L1. For clarity, the control has been omitted in A, being identical with the curve in B (O).

poly(U) has been found with several preparations of EF-G prepared by standard procedures (see Miniprint). Following a proposal by Leberman et al. (37), we have also made several preparations of EF-G using buffer systems containing sodium azide. However, even after prolonged dialysis against azide-free buffer, the resulting EF-G was irreversibly damaged in our hands. Its activity in poly(Phe) synthesis was reduced to less than 5%, the stimulation of the EF-G GTPase by tRNA at 160 mM K\textsuperscript{+} to approximately one tenth. This loss of activity is not apparent in "standard" EF-G GTPase assays not employing tRNA and mRNA or in binding of EF-G-GDP-fusidic acid to ribosomes, assays routinely used to detect EF-G during preparation. Incubation with e.g. 10 mM 2-mercaptoethanol or 2 mM dithiothreitol restored the stimulation of the EF-G GTPase reaction by tRNA to only 20 to 35% of that of control EF-G prepared by standard procedures.

**DISCUSSION**

The availability of *E. coli* mutants lacking one ribosomal protein at a time greatly facilitates the task of assigning functions to these proteins. Reconstitution of activity then requires the incorporation of the missing protein only. For mutants MV17-10 and RD19, Dabbs et al. (38) have shown that protein L1 is missing both in the 50S subunits and the supernatant; likewise, no evidence was found for a drastically altered protein L1. These mutants grow at approximately half the rate of wild type *E. coli* (11). Subramanian and Dabbs (11) have shown that polypeptide synthesis *in vitro* is slowed down accordingly, to about 40 to 60%, and that it can be fully restored by adding pure protein L1.

The present study suggests that two of the tested partial reactions of polypeptide synthesis are responsible for this effect: i.e. binding of peptidyl-tRNA (or tRNA) to the ribosomal P site and the coupled stimulation by tRNA and cognate mRNA of the EF-G GTPase reaction.

Protein L1 has been localized on the 50S subunit by immunoelectron microscopy using wild type, RD19, and MV17-10 50S subunits (38). The binding site for EF-G has been similarly defined (39). As Fig. 7 (40) shows, L1 maps on the wide lateral protuberance opposite to the L7/L12 stalk (41). In the 70S ribosome, the head region of the 30S subunit, where most functional sites have been found, it positioned between the wide lateral protuberance and the central protuberance (1, 42). The binding site for EF-G as determined by immunoelectron microscopy (39) maps close to the origin of the L7/L12 stalk, far away from protein L1. On the other hand, Maassen and Möller (43), using a photochemical cross-linking reagent spanning about 10 Å, have shown direct interaction between EF-G and L1.

From the present results, this protein appears to be functionally important for binding of tRNA to the ribosomal P site. Concerning the stimulation by tRNA of the EF-G

**FIG. 7. Model of the *E. coli* 50S subunit (40) indicating the location of protein L1 and the binding site for EF-G.**
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GTPase reaction, it seems possible that both tRNA and protein L1 act coordinately to anchor EF-G in a more favorable position for GTP hydrolysis. Whether this effect involves direct interaction between EF-G and L1, EF-G and tRNA, or both remains to be elucidated.

Acknowledgements—I am greatly indebted to D. A. R. Subramanian for numerous discussions, for 50 S subunits (from E. coli A19, MV17, 10, and RD19), and for the two-dimensional gel electrophoresis shown in Fig. 1. I thank Dr. D. J. Dijk for generously supplying samples of pure protein L1. I wish to thank Barbara Greuer, Abraham Shevack, Carola Fleischer, and Merold Muller for skillful technical assistance.

REFERENCES

1. Chambloss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M. (eds) (1980) Ribosomes, University Park Press, Baltimore
2. Weissbach, H., and Pestka, S. (eds) (1977) Molecular Mechanisms of Protein Biosynthesis, Academic Press, New York
3. Moller, W. (1974) in Ribosomes (Nomura, M., Tissières, A. and Lengel, P., eds) pp. 711–731, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
4. Parmeggiani, A., and Sander, G. (1981) Mol. Cell. Biochem. 35, 129–158
5. Hamp, H., Schulze, H., and Nierhaus, K. H. (1981) J. Biol. Chem. 256, 2284–2288
6. van Duijn, J., and van Knippenberg, P. H. (1974) J. Mol. Biol. 84, 185–196
7. Szer, W., and Leffler, S. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 3611–3615
8. Suryanarayana, T., and Subramanian, A. R. (1979) J. Mol. Biol. 127, 41–54
9. Dabbs, E. R. (1977) Mol. Gen. Genet. 151, 261–287
10. Dabbs, E. R. (1980) Mol. Gen. Genet. 177, 271–276
11. Subramanian, A. R., and Dabbs, E. R. (1980) Eur. J. Biochem. 112, 425–430
12. Parmeggiani, A., Singer, C., and Gottschalk, E. M. (1971) Methods Enzymol. 20, 291–302
13. Ara, K.-I., Kawakita, M., and Kaziro, Y. (1972) J. Biochem. 247, 7029–7037
14. Chinali, G., Wolf, H., and Parmeggiani, A. (1977) Eur. J. Biochem. 75, 55–66
15. Gillam, J. C., and Tener, G. M. (1971) Methods Enzymol. 20, 55–70
16. Holmes, W. M., Hard, R. E., Reid, B. R., Rimerman, R. A., and Hatfield, G. W. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1068–1071
17. Kern, D., and Lapointe, J. (1979) Biochimie (Paris) 61, 1257–1272
18. Haenni, A.-L., and Chapeville, F. (1966) Biochim. Biophys. Acta 114, 135–148
19. Dijk, J., and Littlechild, J. (1979) Methods Enzymol. 59, 481–502
20. Sander, G., Marsh, R. C., Voigt, J., and Parmeggiani, A. (1975) Biochemistry 14, 1805–1814
21. Sander, G. (1977) Eur. J. Biochem. 75, 523–531
22. Kyriakopoulos, A., and Subramanian, A. R. (1977) Biochim. Biophys. Acta 474, 308–311
23. de Groot, N., Panet, A., and Lapidot, Y. (1971) Eur. J. Biochem. 23, 521–527
24. Lührmann, R., Eckhardt, H., and Stöffler, G. (1979) Nature (Lond.) 280, 423–425
25. Wurmbach, P., and Nierhaus, K. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2143–2147
26. Lucas-Lenard, J., and Lipmann, F. (1967) Proc. Natl. Acad. Sci. U. S. A. 57, 1050–1057
27. Bodley, J. W., Zieve, P. J., Lin, L., and Zieve, S. T. (1970) J. Biol. Chem. 245, 5656–5661
28. Broit, N., Spears, C., and Weissbach, H. (1971) Arch. Biochem. Biophys. 143, 286–296
29. Sander, G., Parlato, G., Crechet, J.-B., Nagel, K., and Parmeggiani, A. (1978) Eur. J. Biochem. 86, 555–563
30. Conway, T. W., and Lipmann, F. (1964) Proc. Natl. Acad. Sci. U. S. A. 52, 1462–1469
31. Nishizuka, Y., and Lipmann, F. (1966) Arch. Biochem. Biophys. 116, 344–351
32. Modolell, J., and Vazquez, D. (1973) J. Biol. Chem. 248, 488–493
33. Ballesta, J. P. G., and Vazquez, D. (1973) Biochemistry 12, 5063–5068
34. Parmeggiani, A., Sander, G., Marsh, R. C., Voigt, J., Nagel, K., and Chinali, G. (1974) In Lipmann Symposium: Energy, Regulation and Biosynthesis in Molecular Biology (Richter, D., ed) pp. 499–510, Walter de Gruyter, Berlin
35. Chinali, G., and Parmeggiani, A. (1982) Eur. J. Biochem. 125, 415–421
36. Rheinberger, H.-J., Sternbach, H., and Nivhauo, K. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5310–5314
37. Leberman, R., Antonsson, B., Giovanielli, R., Guariguata, R., Schumann, R., and Wittinghofer, A. (1980) Anal. Biochem. 104, 29–36
38. Dabbs, E. R., Ehrlich, R., Hasenbank, R., Schroeter, B.-H., Stöffler-Meilicke, M., and Stoffler, G. (1981) J. Mol. Biol. 149, 553–578
39. Girshovich, A. S., Kurtshakia, T. V., Ovschinikov, Y. A., and Vasiliev, V. D. (1981) FEBS Lett. 130, 54–59
40. Noah, M., Stöffler-Meilicke, M., and Stoffler, G. (1982) in Electron Microscopy, 10th International Congress on Electron Microscopy (Congress Organizing Committee, ed) Vol. 3, pp. 101–105, Deutsche Gesellschaft für Elektronenmikroskopie, Hamburg
41. Strycharz, W. A., Nomura, M., and Lake, J. A. (1978) J. Mol. Biol. 126, 123–140
42. Rastner, B., Stöffler-Meilicke, M., and Stoffler, G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6652–6656
43. Masson, J. A., and Möller, W. (1981) Eur. J. Biochem. 115, 279–285
Ribosomal Protein L1 in EF-G GTPase and tRNA Binding

**Ribosomal Protein L1 from Escherichia coli: its role in the binding of tRNA to the ribosome and in elongation factor G-dependent GTP hydrolysis.**

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**Materials and Methods**

The cells (200 g of dry weight/ml) were grown with 400 μg/ml chloramphenicol (Cm) and extracted with 400 ml of buffer (10 mM imidazole-acetate, pH 7.5; 20 mM MgCl₂, 100 μM KCl, and 1 mM dithiothreitol). After centrifugation at 40,000 rpm for 20 min, the ribosomal solution was dialyzed against ribosome buffer containing 10% glycerol or more recently 10% glycerol and stored at -20°C.

Ribosomal subunits were prepared by two different methods: (1) Sonication centrifugation (10 s, 20,000 rpm) in a Beckman 15 Ti rotor of 4,000 to 7,000 Kd units of MgCl₂-washed 70S ribosomes in a 7 to 38% sucrose gradient containing 3.5 mM MgCl₂, 15 mM imidazole acetate (pH 7.5), and 50 mM KCl. The ribosomal solution was dialyzed against ribosome buffer and stored at -20°C in ribosome buffer containing 10% or more recently 10% glycerol.

(2) Direct sonication centrifugation of the 30S using 5 mM MgCl₂, 10 mM Tris pH 7.5, 100 mM KCl and 7.4% mercaptoethanol as buffer. The 30S subunits used were 70S, were dialyzed against the buffer containing sodium dodecyl sulfate.

The 30S subunits were purchased from Pharmacia and purified by chromatography on nonylacrylamide cellulose (15) followed by polyacrylamide gel chromatography using orioctetlyasglutathione (16). The latter procedure was also used to purify tRNA. The tRNA was released with the repressor T4E-labeling was performed on 3A (9, 10, 21) and 14A (11, 21) respectively, using the method of Sauer and Schlieker (22). The purity of the individual ribosomal subunits was checked on SDS-polyacrylamide gels containing sodium dodecyl sulfate.

Poly(U) poly(A), tRNA and GTP (both as Li salts) were from Boehringer, [3H]-GTP, [3H]-GDP, [3H]-GTP, [3H]-GDP, [14C]chymotryptic 19K digest 14C]chymotryptic digest, [14C]lysyl [14C]lysyl and [3H]-tRNA were from New England Nuclear, W. Germany. Purine protein L1 was a generous gift from Dr. J. Dijk (17).

**Fig. 1.** The EF-Tu GTPase reaction as a function of GTP concentration in the absence of ribosome, 100 mM KC1 and 10 mM MgCl₂. (a) Wild type, (b) EF-Tu-100, (c) EF-Tu-1000. Poly(U) was strictly required in (a), poly(A) had no influence in (b).

**Fig. 2A** shows the binding of EF-G-[3H]-GTP-bound acid to SS ribosomes (filled symbols) or SS plus 30S subunits (open symbols) at 20 mM KC1. Mutant and wild type SS subunits showed similar if not identical behavior both with and without ribosomes. EF-G had no influence in (b).

**Fig. 2B** shows the ratio of EF-G-[3H]-GTP-bound acid to SS ribosomes (filled symbols) or SS plus 30S subunits (open symbols) at 20 mM KC1. Mutant and wild type SS subunits showed similar if not identical behavior both with and without ribosomes. EF-G had no influence in (b).

**Fig. 3A** shows the single rate limiting steps in the EF-G GTPase reaction. (a) EF-G with 10 mM MgCl₂ and 10 mM KC1, (b) EF-Tu with 100 mM KC1 and 10 mM MgCl₂, (c) EF-G with 100 mM KC1 and 10 mM MgCl₂. Poly(U) was strictly required in (a), poly(A) had no influence in (b).

**Fig. 3B** shows the single rate limiting steps in the EF-G GTPase reaction. (a) EF-G with 10 mM MgCl₂ and 10 mM KC1, (b) EF-Tu with 100 mM KC1 and 10 mM MgCl₂, (c) EF-G with 100 mM KC1 and 10 mM MgCl₂. Poly(U) was strictly required in (a), poly(A) had no influence in (b).
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*J. Biol. Chem.* 1983, 258:10098-10103.

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