Studies on the Role of an Escherichia coli 50 S Ribosomal Component in Polypeptide Chain Elongation

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SUMMARY

The requirement for PI, a protein component of the 50 S ribosomal subunit of Escherichia coli shown previously to be essential for the interaction of the translation factors EF-T and EF-G with ribosomes, is minimized under certain conditions in partial reactions that can occur independently of chain elongation. Although very little EF-G binding occurs to PI-deficient ribosomes in the presence of GTP or GDP, significant amounts of EF-G can be bound with S′-guanylylmethylenediphosphonate. EF-T-dependent GTP hydrolysis and EF-G-dependent binding of guanine nucleotides, as well as the associated EF-G-dependent GTPase activity carried out under stoichiometric conditions, were found to be much less dependent on PI in the presence of 20% methanol. These observations indicate that the catalytic sites of the PI-deficient-ribosomes are basically intact, and that PI plays an auxiliary role in enhancing the interaction of the elongation factors with the active sites of the ribosomes. End group analysis indicates that PI contains a minimum of two polypeptides with methionine and serine as NH₂-terminal residues. Correspondence of PI to the ribosomal proteins reported by others is suggested on the basis of polyacrylamide gel pattern analysis.

Polypeptide chain elongation is dependent on a complex interaction between ribosome, a mixture of elongation factors Tu and Ts (EF-T) and elongation factor G (EF-G), GTP, aminoacyl-tRNA, peptidyl-tRNA, and mRNA. The sequential addition of each amino acid residue to the nascent protein can be subdivided into three steps: binding of aminoacyl-tRNA (1–4), peptide bond formation (5), and translocation (6–8). It has become increasingly evident that the 50 S ribosomal subunit has a critical role in each of these reactions. The hydrolysis of GTP associated with enzymatic binding apparently occurs on the 50 S particle (9–12), as does the hydrolysis of GTP associated with translocation (6, 11–20). Similarly, the peptidyltransferase activity has been entirely localized to the larger subunit (18, 21–23).

One approach to the study of these reactions is the restoration of activity to partially disassembled 50 S subunits (11, 12, 16–20, 22–27). Most studies have been carried out with 50 S particles made deficient in proteins by centrifugation in CsCl (16, 17, 20, 29-27), and the resulting particles have been found to be defective in their peptidyl transferase and EF-G′-dependent GTPase activities (16, 17, 20, 22, 23). Similar findings have been made with 50 S particles lacking the 5 S RNA (18).

We recently reported a highly specific extraction of the 50 S subunit (the PI extraction) with NH₄Cl and ethanol, which resulted in the removal of a minimum number of proteins (11, 12). The extracted ribosomes were found to have greatly reduced activity in all functions associated with EF-T and EF-G whereas their peptidyltransferase activity was essentially intact (11, 12). The PI extraction was found to have no functional effect on the 30 S subunit, the active component being entirely of 50 S origin even when 70 S ribosomes were extracted. The current studies represent our continued effort to clarify the ribosomal lesion produced by the PI extraction and to characterize further the extracted proteins.

EXPERIMENTAL PROCEDURE

Materials—[3H]Phenylalanine of specific activity 7500 μCi per μmole, [3H]GDP of specific activity 1430 μCi per μmole, and [3H]GTP of specific activity 5600 μCi per μmole were from New England Nuclear; GDPCP was from Miles Laboratories. Fusidic acid was a generous gift from Dr. Josef Fried. All other materials were obtained as described previously (12).

Methods—Unextracted ribosomes, protamine-treated supernatant, EF-G and EF-T, Phe-tRNA*, and N-acetyl-Phe-tRNA were prepared as described previously (12). The EF-T used here was the Peak 1T factor repurified on hydroxylapatite (12). Binding of [3H]GTP and [3H]GDP was measured by the membrane filter (Millipore) technique (12, 28), GTP hydrolysis was assayed by measuring the amount of radioactivity remaining in solution after adsorption of unhydrolyzed [γ-32P]GTP to charcoal (8, 12), and polyacrylamide gel electrophoresis was performed as described previously (12, 29). Ribosomal subunits were separated by sucrose density gradient centrifugation in a Beckman B15 zonal rotor by the procedure of Elkenberry et al. (30); they were then concentrated by precipitation with ethanol and CsCl (12) after diluting the gradient fractions with an equal volume of water. Cross-contamination of the ribosomal subunit preparations was about 2% for the 30 S subunits and 7% for the 50 S subunits as measured by polyphenylalanine

1 The abbreviations used are: EF-G, elongation factor G; EF-T, a mixture of elongation factors Tu and Ts; GDPCP, S′-guanylylmethylenediphosphonate; PI, protein extracted from 50 S ribosomal subunit with NH₄Cl + ethanol.
synthesis. PI extraction was performed on unextracted ribosomes and 50 S particles, and the extracted protein (PI) was precipitated with acetone as described previously (12). Unless specified, all PI used in these experiments was extracted from 70 S ribosomes. All acyloxy contained 0.01 M MgCl₂, 0.05 M imidazole-HCl (pH 7.4), and 0.012 M 2-mercaptoethanol. Reaction volume was 0.25 ml, unless indicated otherwise. Methanol concentrations are stated on a volume per volume basis. Protein concentrations were determined by the method of Lowry et al. (31) with lysozyme as standard.

**Translocation Assays—**Unextracted ribosomes or PI-deficient ribosomes were initially incubated with poly(U) and radioactive N-acetyl-Phe-tRNA at 37°; EF-T, GTP, and nonradioactive Phe-tRNA were then added, and the incubation was continued at 0°; puromycin was added, and the reaction mixtures were briefly incubated at 37°; finally, the pretranslocation complexes were isolated by sucrose density gradient centrifugation (12, 32, 33). In the translocation assays an initial incubation was carried out in order to reduce the amount of EF-G-independent reaction of peptidyl-tRNA with puromycin (12). Each assay contained, in a volume of 0.48 ml, 0.25 ml of pretranslocation complex; 0.08 M NH₄Cl, 0.25 μmole of puromycin, and 0.020 ml of PI as indicated. The reaction mixtures were then incubated for 10 min at 37°. At this time EF-G and GTP were added, bringing the assay volume to 0.50 ml, and incubation was resumed at 37°. The reactions were stopped, and the samples were processed as described previously (12, 34).

**Binding of EF-G to Ribosomes—**The procedure followed was a modification of that used by other investigators (33, 36). The binding mixtures contained 1.0 mg of ribosomes, 0.01 M NH₄Cl, and GDP, GTP, GDP-PCP, EF-G, and PI as indicated. After a 10-min incubation at 37°, each mixture was layered onto 3.5 ml of 10% sucrose containing 0.05 M imidazole-HCl (pH 7.4), 0.01 M MgCl₂ and NH₄Cl, and 0.012 M 2-mercaptoethanol and centrifuged for 6 hours at 55,000 rpm in a Beckman ultracentrifuge (SW 56 rotor). The supernatant was discarded, and the pellet was quickly washed with a buffer containing 0.01 M MgCl₂, 0.08 M NH₄Cl, 0.05 M imidazole-HCl (pH 7.4), and 0.012 M 2-mercaptoethanol. The pellet was then dissolved overnight in 0.25 ml of the same buffer. The amount of EF-G bound to the ribosomes was determined by measuring the GTPase activity of the redisolved ribosomes without any further addition of EF-G. Each reaction mixture contained 200 μg of ribosomes, 5 nmoles of [γ-33P]GTP, 0.08 M NH₄Cl, and 0.020 ml of PI as indicated. Incubation was for 30 min at 37°.

**Electrofocusing—**The procedure was performed as described in the LKB manual, using Ampholine pH 3 to 10 and pH 4 to 6 in different runs. All solutions contained 6 M urea. PI extracted from 100 to 200 mg of 70 S ribosomes was electrofocused in the 110-ml LKB column. Fractions were assayed for activity in polyphenylalanine synthesis as described previously (12).

**Amino Acid Composition of PI—**After dialysis against water, 1.5 ml of PI extracted from 50 S subunits was lyophilized to dryness. The protein was then dissolved in 2.0 ml of 6 N HCl and 0.002 ml of 2-mercaptoethanol and hydrolyzed at 110° for 18 hours. Amino acid composition was determined on a Beckman model 120C amino acid analyzer.

**RESULTS**

**EF-G-dependent Binding of Guanine Nucleotides to Ribosomes—**We noted previously, in studies carried out with [3H]GTP, that the PI-deficient ribosomes were markedly impaired in their ability to bind guanine nucleotide in the presence of EF-G and fusidic acid (11, 12, 14, 28, 37). In the present study, a more thorough examination of this reaction has been carried out with [3H]GDP, as well as [3H]GTP, as the labeled nucleotides. The results presented in Table I show that the PI-deficient ribosomes bound substantial amounts of labeled nucleotides only when PI was present in addition to EF-G. PI alone did not form a complex with EF-G and GTP or GDP which was detectable by filtration through a Millipore filter. The PI-deficient ribosomes appeared to possess more residual binding activity with [3H]GDP than with [3H]GTP. A marked difference in the effect of the 37° incubation on the binding with [3H]GTP and [3H]GDP was observed with unextracted and reconstituted ribosomes, binding with [3H]GTP being substantially increased at 37° as compared to 0°.

These results suggested that there might be a significant difference in the rates of binding of GDP and GTP to ribosomes. This was in fact observed when the binding reactions at 0° were observed over a 40-min interval (Figs. 1 and 2). With both unextracted and reconstituted ribosomes, binding with [3H]GTP was relatively slow and the reaction was not complete at 40 min (Fig. 1), while binding with [3H]GDP was virtually complete by 1 min (Fig. 2). The PI-deficient ribosomes appeared to bind both GDP and GTP at much slower rates than did either the

**Table I**

**EF-G-dependent binding of guanine nucleotides**

| Additions                        | [3H]GTP | [3H]GDP |
|---------------------------------|--------|--------|
|                                  | 0°     | 37°    |
|                                  | 0°     | 37°    |
| Unextracted ribosomes           | 8.7    | 28.2   |
| PI-deficient ribosomes          | 0.4    | 2.4    |
| PI-deficient ribosomes + PI     | 6.3    | 21.0   |
| PI                             | 0      | 0.1    |

*Fig. 1 (left). Kinetics of [3H]GTP binding. All assays contained, in a final volume of 0.25 ml, 0.01 M NH₄Cl, 10 μg of EF-G, 0.25 μmole of fusidic acid, 100 pmoles of [3H]GTP, and 100 μg of ribosomes, and 0.010 ml of PI as indicated. Incubation was for 10 min at 0 or 37°. The data presented have been corrected for the amount of nucleotide bound with EF-G alone.*

*Fig. 2 (right). Kinetics of [3H]GTP binding. Conditions were identical with those described in Fig. 1, except that [3H]GDP was used instead of [3H]GTP.*
unextracted or the reconstituted ribosomes. The PI-extracted ribosomes again had much more residual binding activity with [3H]GDP than with [3H]GTP: at 40 min the PI-deficient ribosomes had bound about 40% as much radioactivity as the reconstituted ribosomes with [3H]GDP but only about 7% as much with [3H]GTP.

**Binding of EF-G to Ribosomes in Presence of Guanine Nucleotides**—Other investigators have found that EF-G was bound by ribosomes in the presence of GDP, GTP, or GDPCP, the last being particularly effective (35, 36). These results were confirmed in a similar study of the PI-deficient ribosomes (Table II). Although the extracted ribosomes were strongly dependent on PI for the binding of EF-G with GDP and GTP, only minimal dependence was observed with GDPCP. No EF-G activity was detected on the ribosomes in all cases when EF-G was omitted in the binding reaction (data not shown). The extent of dependence on PI for EF-G binding was somewhat variable, however, when GTP was the nucleotide used (compare Experiments I and II).

**Requirement of PI in Translocation**—The PI-deficient ribosomes retain the ability to translocate peptidyl-tRNA, but do so at a much slower rate than either the unextracted or the reconstituted ribosomes (11, 12). Furthermore, the PI-extracted ribosomes are virtually devoid of uncoupled EF-G-dependent GTP hydrolysis (11, 12). It was, therefore, of interest to determine whether the residual translating activity of PI-deficient ribosomes required GTP, and whether the GTP requirement differed in the presence and absence of PI.

The data presented in Table III demonstrate that the PI-deficient ribosomes do require GTP, as well as EF-G, to perform the translocation reaction. In fact, the ribosomes had an almost absolute requirement for GTP, whereas both the unextracted and the reconstituted ribosomes showed some reaction with EF-G alone. This GTP-independent translocation may indicate a contamination of the system with GTP in amounts too low to stimulate the PI-deficient ribosomes.
their activity could be restored with methanol. Since a comparison of the polyacrylamide gel patterns clearly indicates that the β cores lack both the PI proteins and a number of additional proteins as well (cf. the gels in References 12 and 22), we decided to examine the effect of methanol on the EF-G-dependent GTPase activity of the extracted ribosomes. As seen in Table IV, methanol was not able to substitute for PI with either 70 S ribosomes or ribosomal subunits. The effect of methanol was somewhat variable, having a slightly inhibitory effect on hydrolysis with 70 S ribosomes and a small stimulatory effect on the residual hydrolysis with 50 S subunits (6, 15, 17). Table V presents the results of our studies on the effect of methanol on the EF-G-dependent binding of [%H]GDP and [%H]GTP to ribosomes. With unextracted and reconstituted ribosomes, methanol briskly stimulated the binding of [%H]GTP but had little effect on the binding of [%H]GDP (Table V, 38). The alcohol also dramatically stimulated the binding activity of PI-deficient ribosomes, but with both nucleotides. Methanol thus appears to reduce substantially the requirement for PI in guanine nucleotide binding.

When the activity of ribosomal subunits was examined, essentially the same results were obtained (Table V). In contrast to other workers (33, 14), we found that 30 S subunits markedly stimulated the binding of [%H]GTP by 50 S subunits. This difference appears to be due to our use of lower levels of EF-G.2 The stimulation by 30 S particles of the binding of [%H]GTP, however, was not as marked. Methanol enhanced the binding of both nucleotides to unextracted 50 S subunits, but the stimulation of [%H]GTP binding was much greater than the stimulation of [%H]GDP binding. Interestingly, with PI-deficient 50 S ribosomes, methanol stimulated [%H]GDP binding much more than [%H]CTP binding, unless 30 S particles were also present.

1 Unpublished observations.

Table IV

| Additions                                      | Amount of GTP hydrolyzed | –Methanol | +20% Methanol |
|------------------------------------------------|--------------------------|-----------|---------------|
| Experiment I                                   |                          |           |               |
| Unextracted ribosomes                          | 6.7                      | 4.6       |               |
| PI-deficient ribosomes                         | 0.3                      | 0         |               |
| PI-deficient ribosomes + PI                    | 5.9                      | 5.2       |               |
| Experiment II                                  |                          |           |               |
| 30 S                                           | 0.5                      | 0.1       |               |
| 50 S                                           | 3.6                      | 0.1       |               |
| PI-deficient 50 S                              | 0.2                      | 0.8       |               |
| 30 S + 50 S                                   | 14.8                     | 13.6      |               |
| 30 S + PI-deficient 50 S                       | 1.1                      | 2.5       |               |
| 30 S + PI-deficient 50 S + PI                  | 15.6                     |           |               |

When GTP cleavage was examined under conditions identical with those used in the study of [%H]GTP binding, it was found that the GTP bound by the extracted ribosomes in the presence of methanol was hydrolyzed (Table VI). In 20% methanol the requirement for PI in the stoichiometric hydrolysis of GTP in the absence of methanol, and to that observed in the catalytic hydrolysis of GTP, where the requirement for PI was almost complete with and without methanol (Table IV).

Table V

| Additions                                      | Amount of nucleotide bound | –Methanol | +20% Methanol |
|------------------------------------------------|---------------------------|-----------|---------------|
| Experiment I                                   |                           |           |               |
| Unextracted ribosomes                          | 6.9                       | 26.5      | 18.9          | 23.2          |
| PI-deficient ribosomes                         | 0.1                       | 16.1      | 0.6           | 15.3          |
| PI-deficient 50 S                              | 4.5                       | 20.8      | 15.2          | 18.3          |
| PI-deficient 50 S + PI                         | 4.2                       |           | 14.6          |

Table VI

| Additions                                      | Amount of GTP cleaved | –Methanol | +20% Methanol |
|------------------------------------------------|-----------------------|-----------|---------------|
| Unextracted ribosomes                          | 3.7                   | 19.9      |
| PI-deficient ribosomes                         | 0.3                   | 11.6      |
| PI-deficient ribosomes + PI                    | 4.0                   | 19.6      |

1 Unpublished observations.
TABLE VII

Effect of poly(U) on EF-T-dependent GTP hydrolysis by PI-deficient ribosomes

| Additions | Amount of GTP hydrolyzed |
|-----------|-------------------------|
|           | -Poly(U) | + Poly(U) |
| Experiment I |         |           |
| Unextracted ribosomes | 6.5 | 13.3 |
| PI-deficient ribosomes | 0.8 | 2.4 |
| PI-deficient ribosomes + PI | 4.6 | 9.9 |
| Experiment II |          | |
| 30 S | 0.5 | 1.2 |
| 50 S | 1.1 | 1.1 |
| 30 S + 50 S | 3.9 | 17.8 |
| 30 S + PI-deficient 50 S | 1.1 | 5.4 |
| 30 S + PI-deficient 50 S + PI | 3.5 | 10.7 |

Effects of Poly(U) and Methanol on PI Requirement in EF-T-dependent Hydrolysis of GTP—Since Sopori and Lengyel (20) recently reported that the EF-T-dependent GTPase activity of β-cores of 50 S subunits was not dependent on PI or the split β proteins in the presence of 30 S subunit and poly(U), whereas we observed a strong requirement for PI in the absence of poly(U) (11, 12, 39), we examined further the PI requirement for this reaction. As seen in Table VII (Experiments I and II) the addition of poly(U) to the system did not abolish the requirement for PI. We found, moreover, that EF-T-dependent GTP hydrolysis occurred only in the presence of both ribosomal subunits, whether or not poly(U) was included in the assay system (9, 10, 20).

As reported elsewhere (38), methanol strongly stimulates the EF-T-dependent GTPase activity. It was of interest, therefore, to examine the effect of methanol on the requirement for PI in this reaction. As shown in Table VIII, a requirement for PI was still seen in the EF-T-dependent GTPase activity, but it was definitely less than that observed in the absence of methanol (11, 12; Table VII). No GTP hydrolysis was detectable without methanol under the conditions employed in the experiment. Some EF-T-dependent GTPase activity was found with the unextracted 50 S subunit alone in the presence of methanol (Table VIII, Experiment II; see also Reference 9), but, interestingly, this reaction was still strongly dependent on PI. The PI-deficient 30 S subunits were more active in the presence of 30 S particles without PI than they were when supplemented with PI but without 30 S subunits (compare Lines 4 and 5 of Experiment II).

Purification, Characterization, and Analysis of PI—Since PI protein has been shown to electrophorese as two bands on polyacrylamide-urea gels at pH 4.5 (12), several attempts were made to separate preparatively the two bands of PI. Fig. 4 illustrates the results obtained by electrophocusing, using PI extracted from

TABLE VIII

Effect of methanol on EF-T-dependent GTPase activity

| Additions | Amount of GTP cleaved |
|-----------|-----------------------|
| Experiment I |                  | |
| Unextracted ribosomes | 700 | |
| PI-deficient ribosomes | 270 | |
| PI-deficient ribosomes + PI | 630 | |
| PI-deficient ribosomes + PI, without methanol | 0 | |
| Experiment II |              | |
| 30 S | 0 | |
| 50 S | 160 | |
| PI-deficient 50 S | 20 | |
| PI-deficient 50 S + PI | 120 | |
| 30 S + 50 S | 750 | |
| 30 S + PI-deficient 50 S | 380 | |
| 30 S + PI-deficient 50 S + PI | 700 | |
Therefore, performed directly on PI extracted from isolated 50 S particles, since these extracts show minimal contamination by other ribosomal proteins on polyacrylamide gel electrophoresis (12). Two NH_2-terminal amino acids were found, methionine and serine, in a ratio of about 2:1 (data not presented), indicating that PI contained a minimum of two polypeptide chains. Phenylthiohydantoin-serine, however, is unstable under the conditions of the analysis, and the value for serine is therefore only a minimal estimate (47).

**DISCUSSION**

The results presented here show that the PI-deficient ribosomes have, under appropriate conditions, a reduced requirement for FI in their interactions with guanine nucleotides and EF-G and EF-T. In the absence of PI, methanol was found to enhance substantially the participation of the extracted ribosomes in the EF-G-dependent binding of [3H]GTP and [3H]GDP and the associated stoichiometric hydrolysis of GTP. Methanol was also found to stimulate the EF-T-dependent GTPase activity of the PI-deficient ribosomes. The extracted ribosomes retained a slow but substantial activity in the EF-G-dependent binding of [3H]GDP, and they bound significant amounts of EF-G in the presence of GDP:C.

These findings complement a number of observations made in an earlier study of the role of PI in polypeptide chain elongation (11, 12). It was then demonstrated that, although PI was required for a maximum reaction rate, a substantial proportion of the extracted ribosomes eventually participated in the enzymatic binding and translocation reactions in the absence of PI. Moreover, both reactions required the appropriate factor and GTP (Table III), and the binding reaction was accompanied by peptide bond formation (12).

Although it is possible that the PI-deficient ribosomes functioned in the absence of PI because of the catalytic utilization of a small amount of contaminating PI, this is not considered likely for the following reasons. (a) PI is found on both 70 S

**Table IX**

Amino acid composition of PI

| Amino acid | PI | Kaltschmidt et al. (40) | Mora et al. (45) | Moller et al. (46) |
|------------|----|------------------------|-----------------|-------------------|
|            | mole % | mole % | mole % | mole % |
| Asp        | 6.4 | 6.6 | 6.7 | 6.9 | 6.9 |
| Thr        | 2.8 | 3.0 | 2.9 | 2.8 | 2.8 |
| Ser        | 4.6 | 5.1 | 5.1 | 5.3 | 4.4 |
| Glu        | 15.4 | 14.9 | 14.1 | 15.0 | 13.1 |
| Pro        | 2.1 | 1.7 | 1.9 | 2.2 | 2.2 |
| Gly        | 7.0 | 6.9 | 7.2 | 7.6 | 6.6 |
| Ala        | 22.1 | 24.0 | 23.6 | 23.6 | 19.8 |
| Val        | 12.2 | 13.5 | 13.1 | 11.5 | 11.9 |
| Met        | 2.0 | 2.6 | 2.3 | 2.4 | 2.3 |
| Ile        | 4.1 | 3.4 | 4.0 | 3.0 | 4.7 |
| Leu        | 7.6 | 8.8 | 6.4 | 6.9 | 7.8 |
| Tyr        | 0.1 | 0.3 | 0.4 | 3.8 | 0.8 |
| Phe        | 1.9 | 1.5 | 1.6 | 1.9 | 3.8 |
| His        | 0.2 | 0.0 | 0.1 | 0.8 | 0.8 |
| Lys        | 10.6 | 10.1 | 9.4 | 10.0 | 9.2 |
| Arg        | 1.0 | 0.8 | 1.0 | 0.9 | 2.7 |

*Fig. 5. Polyacrylamide gel electrophoresis of electrofocused PI. PI from 100 mg of 70 S ribosomes was purified by electrofocusing as described in Fig. 4, except that Ampholine, pH 4 to 6, was used. The pooled fractions were made 0.5 M in KCl and passed through a Sephadex G-25 column (35 × 2.4 cm) equilibrated with 0.5 M KCl, 0.01 M imidazole-HCl (pH 7.4), and 0.002 M 2-mercaptoethanol. Both purified and crude PI were subjected to electrophoresis as described previously (12, 29). The gel with the crude PI appears on the left, that with the purified PI on the right. The broad fuzzy band in the middle of the gel on the right is probably due to residual Ampholine.*
ribosomes and native 50 S subunits; (b) PI is not readily extractable from the ribosomes by high concentrations of NH$_4$Cl alone; and (c) PI is required by the ribosomes for the entire duration of chain elongation. We therefore conclude that the catalytic sites involved in aminoacyl-tRNA binding, in translocation, and in the hydrolysis of GTP associated with these two reactions remain basically intact in the PI-deficient ribosomes, and that PI plays an auxiliary role in these reactions, enhancing the interaction of the ribosomes with the elongation factors and GTP.

Indirect support for this conclusion comes from the studies of Ballesta et al. (17) and Sopori and Lengyel (20) with the $\beta$-cores obtained by extraction of 50 S particles with CsCl (22). Although lacking a number of proteins still retained by the PI-deficient ribosomes (12, 22), the $\beta$-cores appear to possess catalytic activities lost by the PI-deficient ribosomes. Ballesta et al. (17) restored catalytic EF-G-dependent GTP hydrolysis to the $\beta$-cores with methanol, while Sopori and Lengyel (20) found them fully active in the coupled S$_3$(Tu)-dependent hydrolysis of GTP (48). These activities both require PI if PI-deficient ribosomes are used, as shown here. It is possible that a more extensive extraction of proteins from the 50 S particle eliminated the requirement for PI.

Although we were unable to obtain a homogeneous preparation of PI, we can correlate the proteins of PI with the proteins described by others on the basis of polyacrylamide gel pattern and amino acid composition. The two bands of PI clearly correspond to the L7 and L12 proteins reported by Wittmann's group (40-42) to have identical amino acid compositions, molecular weights, and immunological properties. DeLucia and Traut (43) and Traut et al. (44), on the other hand, obtained four bands for the proteins corresponding to PI by a modification of the polyacrylamide gel used; all four components were found to have similar molecular weights and were immunologically cross-reactive (44). PI also has significant similarities to the A protein described by Møller and his co-workers (16, 46, 49, 50), although we obtained different results on NH$_2$-terminal analysis and a significantly different isoelectric point than that reported by these authors.

Despite the similarity of molecular weights and immunological cross-reactivity of the components corresponding to PI, our observation that methionine and serine are NH$_2$-terminal residues of PI indicates that PI is heterogeneous and must consist of at least two separate polypeptide chains. Moreover, the amount of PI extractable from the 50 S ribosomal subunit also tends to suggest a multiplicity of some kind. We have consistently found that the amount of PI extracted corresponded to about 3 to 3.5% of the mass of the 50 S particle. This would mean that 3 to 5 protein molecules are extracted per 50 S particle, assuming a molecular weight of 12,000 to 23,000 (19, 41, 43-46, 49).

One of the more interesting properties of the PI-deficient ribosomes is their slow translocating activity. It was shown previously that PI-independent translation could be minimized at low levels of EF-G (12). We have shown here that PI-independent translocation can also be minimized at low concentrations of GTP. It should therefore be possible to virtually eliminate PI-independent translocation by using very low levels of EF-G and GTP, thus making translocation dependent on the addition of a ribosomal protein component. This feature of the PI-deficient ribosomes may prove useful in studying the molecular mechanism of translocation.

Finally, the kinetics of EF-G-dependent binding of guanosine nucleotide with [H]GTP appears to be slower than that observed previously by others (28, 51). The reason for this is not clear. It may be due to a difference in the preparation of EF-G or ribosomes, or in the experimental conditions. Further studies are being carried out to resolve this problem.

Acknowledgments—The authors are indebted to Mrs. C. Edelstein and Mr. S. Boxer for the amino acid composition analysis and to Drs. R. Heinrikson, C. Noyes, and H. Kingston for the Edman degradation of PI which was carried out on a Beckman model 880 Sequencer.

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*J. Biol. Chem.* 1972, 247:6810-6817.

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