POLYPEPTIDE CHAIN INITIATION IN E. COLI: ISOLATION OF HOMOGENEOUS INITIATION FACTOR F2 AND ITS RELATION TO RIBOSOMAL PROTEINS

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Communicated January 22, 1969

Abstract.—Previous work has shown that F2, one of several ribosomal factors involved in polypeptide chain initiation, functions in the binding of formylmethionyl-transfer RNA (fMet \( \sim \) tRNA\(_f\)) to a messenger RNA-ribosome complex. F2 was isolated from 1.0 \( M \) ammonium chloride washes of E. coli Q13 ribosomes as a protein homogeneous on polyacrylamide gel electrophoresis at both pH 4.5 and 7.8. Its molecular weight is approximately 80,000. Comparison of electrophoretic patterns of ribosomal proteins from NH\(_4\)Cl-washed and unwashed ribosomes and F2, at pH 4.5, shows that F2 corresponds to the slowest-moving component of the proteins derived from unwashed ribosomes. This component is missing from the NH\(_4\)Cl-washed ribosomes. The activity of F2 is stimulated by two additional factors, initiation factor F1 and a factor(s) present in a narrow ammonium sulfate fraction of the ribosomal NH\(_4\)Cl wash. The nature of the latter is unknown.

The formation of a complex involving the 30S ribosomal subunit, fMet \( \sim \) tRNA\(_f\), and messenger RNA is an obligatory step in the initiation of protein synthesis in E. coli. Ribosomal initiation factors and GTP are required for this reaction.\(^1\) Studies made to ascertain the specific role of individual initiation factors showed that F2 functions in the binding of mRNA to the ribosomes and that F2 functions in the binding of fMet \( \sim \) tRNA\(_f\) to the mRNA-ribosome complex,\(^2\) but the precise mechanism by which the initiation factors and GTP function in chain initiation is poorly understood. Recently we have found\(^3\) that the activity of F2, one of the initiation factors, is inhibited by SH-binding reagents. Interestingly, either GTP or 30S ribosomes can protect F2 against inactivation by these agents, suggesting that F2 has "essential" SH groups that become masked upon formation of a GTP-F2 complex or upon binding to ribosomes. In the present paper we wish to report on the isolation of electrophoretically homogeneous F2 and its relation to the ribosomal proteins. The involvement of SH groups in F2 activity and the formation of a GTP-F2 complex will be the subject of an ensuing communication.

Materials and Methods.—Ribosomes and ribosomal proteins: Purified 1.0 \( M \) NH\(_4\)Cl-washed E. coli Q13 ribosomes were prepared as previously described.\(^4\) The ribosomal pellet was suspended in a buffer containing 250 mM NH\(_4\)Cl, 5 mM magnesium acetate, 20 mM Tris-HCl buffer (pH 7.8), 0.5 mM dithiothreitol (DTT), and 50% glycerol (v/v) at a concentration of approximately 1000 A\(_{260}\) units/ml and stored at \(-10^\circ\). Under these conditions, the ribosomes kept their activity for at least one month. "Unwashed" ribosomes were prepared by suspending the crude ribosomal pellet in a buffer containing 20 mM Tris-HCl (pH 7.8), 50 mM NH\(_4\)Cl, 10 mM magnesium acetate, and 1 mM DTT, centrifuging at 105,000 \( \times \) g for 4 hr at \(2^\circ\), and repeating the buffer washing once more.
We are indebted to Miss Martha J. Miller for this preparation. Since ribosomes that have not been washed with 1.0 M NH₄Cl lose their activity rather rapidly (owing to absence of proteolytic enzymes?), they were used immediately after assay had shown high amino acid incorporation activity in the absence of added initiation factors and no stimulation by this addition.

Proteins were prepared for gel electrophoresis from purified and from "unwashed" ribosomes by extraction with 66% acetic acid (according to Waller) followed by dialysis, for 4 hr at 4°C, against β-alanine (0.35 M) -acetate buffer, pH 4.5, to remove excess acetic acid.

F₄ assay: The virtually absolute requirement of F₄ for the AUG-dependent binding of fMet-tRNAₐ to purified ribosomes at low Mg²⁺ concentrations was the basis for assay of F₄ activity. In the presence of F₄, binding is stimulated by the initiation factor F₁, and, as we found, is further stimulated by an ammonium sulfate fraction of the 1.0 M NH₄Cl ribosomal wash. This fraction will be referred to as the supplemental (S) fraction. It can be replaced by purified preparations of the initiation factor F₁ and it itself contains F₄ activity. Although it has previously been referred to as F₄, it is better to designate it as fraction S until a clear-cut decision as to its nature can be made. F₁ and fraction S were always added in excess to F₄ assay samples. Unless otherwise stated, all assay samples contained, in a final volume of 0.05 ml, Tris-HCl buffer, pH 7.2, 50 mM; NH₄Cl, 150 mM; magnesium acetate, 3.5 mM; GTP, 0.2 mM; ApUpG (AG), 0.04 A₄₆₅ₐₕ unit; fMet-tRNA labeled with ¹⁴C-methionine, 20 μmoles (4800 cpm); purified ribosomes (50S and 30S subunits), 2.5 A₂₆₀ units; F₄ with 5.5 μg of protein; fraction S with 6 μg of protein; F₁, 0.0005-0.003 units; and glycerol, 2% v/v. Incubation was for 15 min at 25°C; then binding was determined by the procedure of Nirenberg and Leder. The Millipore filters were washed with a buffer containing 0.1 M Tris-HCl, pH 7.2, NH₄Cl (50 mM), and magnesium acetate (5 mM). They were then dried and inserted into scintillation vials. Ribosome-bound radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. The scintillation fluid contained 6 gm of 2,5-diphenyloxazole (PPO) and 100 mg of pbis 2-(5-phenyloxazolyl)benzene (POPOP) per liter of toluene. Net binding values were calculated by subtracting the small blanks obtained in the absence of F₄.

Under the above assay conditions, the amount of fMet-tRNA bound was proportional to the concentration of F₄ within a tenfold range of concentrations, as seen in Figure 1. One unit was defined as the amount of F₄ causing the binding of 1 μmole/sample in 15 min. Specific activity is expressed in units/mg protein. Protein was determined spectrophotometrically.²

Assay components: (1) F₁ was prepared from the 1.0 M NH₄Cl ribosomal wash² with starting 100 gm of frozen E. coli Q13 cells. After the ribosomes had been stirred overnight in a buffer containing 1.0 M NH₄Cl, 20 mM Tris-HCl (pH 8.1), 2 mM magnesium acetate, and 10 mM mercaptoethanol, the suspension was centrifuged at 105,000 g for 4.5 hr. The supernatant was dialyzed for 6 hr against a buffer containing 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 10 mM mercaptoethanol. The dialyzed solution was loaded onto a column (1.7 × 35 cm) of DEAE-cellulose previously equilibrated with the above buffer, and the column was then washed with this buffer at a rate of about 60 ml/hr. Fractions (about 1.8 ml) were collected. Those containing the peak of F₁ activity (#68 to 75), as assayed by stimulation of F₁ activity in the above assay (without fraction S), were pooled and stored at 4°C.

(2) Fraction S was an ammonium sulfate fraction (at 0.55-0.70 saturation) of the supernatant remaining after ammonium sulfate precipitation of F₁ from the NH₄Cl ribosomal wash (see F₁ purification, step 2). The precipi-
tate was dissolved in a small volume of a buffer containing 20 mM Tris-HCl (pH 7.8), 20 mM NH₄Cl, 20 mM magnesium acetate, 2 mM DTT, and 50% glycerol (v/v), and stored at -10°C. This fraction was suitably diluted before use in an identical buffer, except for a lower (5%) glycerol content.

(3) Crude tMet-\(^{14}C\)tRNA\(_f\), labeled with \(^{14}C\) in the methionine residue, was prepared as previously, except that dialyzed \(E.\ coli\) Q13 supernatant was used as a source of tMet-\(~tRNA\) synthetase and formyl transferase. When required, contaminating GTP was removed by gel filtration on Sephadex G-50.

(4) The dinucleoside diphosphate ApUpG (AUG) was a product of the Miles Laboratories, Inc.

Other assays: Purified F\(_2\) was assayed for contamination with other activities as follows: Exonuclease was assayed by Miss Martha J. Miller with \(^{14}C\)-polyn as substrate, as described by Salas et al. Elongation factors T and G were kindly assayed by Dr. Julian Gordon, Rockefeller University. GTPase activity was assayed in the absence and presence of ribosomes, as described by Kolakofsky et al., with GTP labeled with \(^{32}P\) in the \(\gamma\) phosphate. This was diluted with unlabeled GTP to a specific radioactivity of 355 cpm/\(\mu\)mole.

Gel electrophoresis: Disc electrophoresis on polyacrylamide gels was run either at pH 4.5 or 7.8. Electrophoresis at pH 4.5 was carried out by the procedure of Reisfeld et al. with some modifications. All stock solutions used for preparing the gels contained 8.0 M urea, deionized as described by Duesberg et al. The final concentrations of acrylamide and bisacrylamide in the separating gels were 7.5% and 0.3%, respectively. The corresponding concentrations in stacking gels were 3.5% and 0.42%. Electrophoresis was conducted for 4 hr at 25°C and 90 v in \(\beta\)-alanine (0.35 M)-acetate buffer, pH 4.5. The column length was 11 cm.

For electrophoresis at pH 7.8, the separating gel contained 0.375 M Tris-HCl buffer (pH 7.8), 0.06% (v/v) tetramethylethylenediamine (TEMED), 7.5% acrylamide, and 0.2% bisacrylamide (w/v). The stacking gel contained 0.046 M Tris-HCl buffer (pH 6.8), 0.04% TEMED, 3.5% acrylamide, and 0.2% bisacrylamide. The column length was 7 cm. Electrophoresis was conducted for 7 hr at 25°C and 200 v in Tris (4.96 mM) -glycine (38.6 mM) buffer, pH 8.3. After electrophoresis the gels were stained with amido black and then destained with 7% acetic acid.

Miscellaneous: Dithiothreitol (DTT) was obtained from Nutritional Biochemicals. Sephadex G-200 (Pharmacia, particle size 40-120 \(\mu\)) was treated prior to use with 2 mM EDTA, and the EDTA was removed by a thorough washing with water. DEAE-SH cellulose (Serva, 0.83 meq/gm) was washed successively with 0.2 N NaOH, water, 0.2 N HCl, water, and suspended in a buffer containing 20 mM NH₄Cl, 20 mM Tris-HCl, (pH 7.8), 0.2 mM magnesium acetate, 2 mM DTT, and 5% glycerol (v/v). Hydroxylapatite (Hyapatite C, lot #6338) was a product of the Clarkson Chemical Co. Other reagents were as used in previous work.

Results and Discussion.—Purification of F\(_2\): F\(_2\) was purified from the 1.0 M NH₄Cl-wash of \(E.\ coli\) Q13 ribosomes. All operations were carried out at 0-4°C unless otherwise stated.

Step 1. \(NH₄Cl\) ribosomal wash: About 600 gm of frozen cells were processed each day. The cells were thawed and evenly suspended in 600 ml of a buffer (buffer A) containing 20 mM Tris-HCl, (pH 7.8), 10 mM magnesium acetate, and 2 mM DTT. 100-ml portions of this suspension were sonicated for two minutes in a Benson model S-125 sonic oscillator. The temperature of the suspension after sonic disintegration never exceeded 9°C. The suspension was centrifuged for 20 minutes at 30,000 \(g\) in a refrigerated Serval RC 2-B centrifuge. The supernatant was stirred with DNase (3 \(\mu\)g/ml) in the cold for a few minutes and centrifuged as above (S30 extract). The S30 extract
was centrifuged for 2.5 hr at 160,000 g in the #50 rotor of the Spinco model L ultracentrifuge. After a thorough draining, the ribosomal pellets were suspended in 250 ml of a buffer (buffer B) containing 1.0 M NH₄Cl, 20 mM Tris-HCl buffer (pH 7.8), 2 mM magnesium acetate, and 2 mM DTT, and stirred overnight. The suspension was centrifuged at 160,000 g as outlined above, and the resulting supernatant (1.0 M NH₄Cl wash) was stored at 2–4°. A total of 4 kg of frozen cells was worked up in this manner, yielding 1.664 ml of solution with 12.4 mg of protein/ml.

**Step 2. Ammonium sulfate fractionation:** About one third of the 1.0 M NH₄Cl wash was processed each time. Finely powdered ammonium sulfate (19.4 gm/100 ml) was added slowly while the mixture was being stirred. After 20 to 30 minutes, the mixture was centrifuged for 10 to 15 minutes at 30,000 g in the refrigerated Sorvall centrifuge, and the precipitate was discarded. More ammonium sulfate 5.7 gm/100 ml of supernatant was added. The mixture was centrifuged as above. The precipitate (0.35–0.45 saturation) was dissolved in a minimal volume of a buffer (buffer C) containing 20 mM NH₄Cl, 20 mM Tris-HCl (pH 7.8), 0.2 mM magnesium acetate, 2 mM DTT, and 5 per cent glycerol (v/v), and was stored at −10°. Prior to step 3, the pooled solutions were diluted with buffer C to a protein concentration of 20 mg/ml, dialyzed for three hours against several changes of buffer C and then dialyzed for another hour against the same buffer, except that the pH of the Tris-HCl was 7.0. A precipitate that formed during dialysis was removed by centrifugation and discarded. This step yielded 149 ml of solution with 15.2 mg of protein/ml. The ammonium sulfate supernatant (0.45 saturation) was used for the preparation of fraction S as described in Materials and Methods.

**Step 3. Adsorption and elution from calcium phosphate gel and ammonium sulfate fractionation:** The solution from the previous step was diluted to a protein concentration of 5 mg/ml with buffer C (pH 7.0). Calcium phosphate gel (30 mg dry weight/ml) was added in the proportion of 1 mg gel/mg protein and the mixture was stirred for 30 minutes. The gel was centrifuged down, suspended in 180 ml of 0.01 M potassium phosphate buffer, pH 7.5 (containing 10 mM mercaptoethanol), with the aid of a glass homogenizer and stirred for 20 minutes. The suspension was then centrifuged. The precipitated gel was successively eluted, as described above, with 80 ml of 0.03, 0.2, and 0.3 M potassium phosphate buffer (pH 7.5) containing 10 mM mercaptoethanol. The eluates containing F₂ of highest specific activity (usually 0.03 and 0.2 M phosphate) were pooled. A 0.35–0.45 saturation ammonium sulfate fraction was obtained from the pooled eluates as described in step 2. The precipitate was dissolved in a minimal volume of a buffer (buffer D) containing 20 mM Tris-HCl (pH 7.8), 20 mM, NH₄Cl, 0.2 mM magnesium acetate, 2 mM DTT, and 50 per cent glycerol (v/v). This yielded 21 ml of solution with 22 mg of protein/ml. It was stored at −10°.

**Step 4. Sephadex G-200 chromatography:** The solution from the previous step was put on a Sephadex G-200 column (2.7 × 75 cm) previously equilibrated with buffer C. The column was then washed with the same buffer at a flow rate of about 14 ml/hr, and 3.0-ml fractions were collected. The fractions of
highest specific activity (82 through 100) were pooled, yielding 55.5 ml of solution with 1.8 mg of protein/ml.

Step 5. **DEAE-cellulose chromatography:** The solution from the above step was immediately loaded onto a column (2.2 \times 45 cm) of DEAE-cellulose previously equilibrated with buffer C. Elution was carried out at a flow rate of 16 ml/hr with a 0.02-0.3 M linear gradient of NH₄Cl in buffer C (300 ml of buffer C in the mixer and 300 ml of 0.3 M NH₄Cl in buffer C in the reservoir). Fractions (about 2.6 ml) were collected, and those containing F₂ of highest specific activity (150 through 160) were pooled and immediately dialyzed overnight against a solution (adjusted to pH 7.5) containing 50 mM Tris-HCl, 20 mM NH₄Cl, 2 mM magnesium acetate, 10 mM mercaptoethanol, and ammonium sulfate at 0.70 saturation (buffer E). The precipitated protein was collected by centrifugation, dissolved in a minimal volume of buffer D, and stored at \(-10^\circ\). This yielded 0.6 ml of solution with 17.5 mg of protein/ml.

Step 6. **Hydroxylapatite chromatography:** The solution from step 5 was diluted to 2.5 ml with buffer C and dialyzed for 2.5 hours against several changes of the same buffer. The dialyzed solution was transferred to a column (0.8 \times 19 cm) of hydroxylapatite previously equilibrated with buffer C containing, in addition, 1.0 mM potassium phosphate buffer, pH 7.5. Elution was carried out at a flow rate of about 2.0 ml/hr, with a 0.02-0.2 M linear gradient of potassium phosphate in buffer C (50 ml of 0.02 M potassium phosphate, pH 7.5 in buffer C in the mixer and 50 ml of 0.2 M potassium phosphate, pH 7.5, in buffer C in the reservoir). Fractions (of 1.1 ml) were collected. Fractions 24 through 36 were pooled and immediately dialyzed overnight against buffer E. The precipitate was dissolved in a minimal amount of buffer D, yielding 0.5 ml of solution with 4.4 mg of protein/ml, and stored at \(-10^\circ\). A summary of the purification procedure is given in Table 1.

From the data in Table 1, it can be calculated that 220 mg (about 1%) of the protein (20,640 mg) in the ribosomal wash at step 1 is F₂ protein. The

**Table 1. Purification of F₂.*

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Protein (mg)</th>
<th>Units†</th>
<th>Specific activity‡</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NH₄Cl-wash of ribosomes</td>
<td>1,664</td>
<td>20,640</td>
<td>3,483</td>
<td>0.17</td>
<td>100</td>
</tr>
<tr>
<td>2. (NH₄)₂SO₄ fractionation</td>
<td>149</td>
<td>2,265</td>
<td>1,314</td>
<td>0.58</td>
<td>38</td>
</tr>
<tr>
<td>3. Adsorption and elution from Ca₃(PO₄)₂ gel</td>
<td>21</td>
<td>455</td>
<td>602</td>
<td>1.34</td>
<td>17</td>
</tr>
<tr>
<td>4. Sephadex G-200 chromatography</td>
<td>55</td>
<td>100</td>
<td>330</td>
<td>3.3</td>
<td>9.5</td>
</tr>
<tr>
<td>5. DEAE-cellulose chromatography</td>
<td>0.6</td>
<td>10.5</td>
<td>107</td>
<td>10.2</td>
<td>3.2</td>
</tr>
<tr>
<td>6. Hydroxylapatite chromatography</td>
<td>0.5</td>
<td>2.2</td>
<td>35.7</td>
<td>16.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* From 4 kg of frozen *E. coli* Q13 cells.
† 1 unit = 1.0 mmole of fMet(14C)-tRNA bound under standard assay conditions (15 min, 25°).
‡ Units/mg protein.
reasons for the very low F₂ yields are not clear. A preparation obtained by a slightly different procedure gave similar yields.

Properties and purity of F₂: The A₂₈₀/A₂₆₀ ratio of the step 6 preparation was 1.8 (A₂₈₀/A₂₆₀ at step 1, about 0.6). Disc electrophoresis on polyacrylamide gel at pH 4.5 and 7.8 (Fig. 2) showed that step 6 F₂ is homogeneous. This was not the case at step 5.

Gel filtration data suggested a molecular weight of about 100,000. A preliminary determination of the molecular weight of F₂ was carried out by the meniscus depletion method of Yphantis, which employs the Spinco UV scanning

![Fig. 2. - Polyacrylamide gel electrophoresis of F₂ (step 6). (A) pH 7.8; F₂, 20 μg. (B) pH 4.5; F₂, 8 μg. Origin at top in both cases. In (A) the anode, and in (B) the cathode, is at the bottom.](image)

![Fig. 3.-Polyacrylamide gel electrophoresis (pH, 4.5) of F₂ and of proteins from 1.0 M NH₄Cl-washed and unwashed 70S ribosomes. Origin at top, cathode at bottom. (A) Split gel with (a) protein (40 μg) from NH₄Cl-washed ribosomes or (b) protein (40 μg) from unwashed ribosomes. (B) F₂ (8 μg). (C) Protein (80 μg) from unwashed ribosomes. (D) Protein (80 μg) from NH₄Cl washed ribosomes. By numbering the first few protein bands from the top it is shown that in A(a) and D, band 1 (F₂) and band 4 are missing from the washed ribosomes, and bands 2 and 3 are fainter.](image)
system at 280 m\(\mu\). A determination at one \(F_2\) (step 6) concentration (0.27 mg/ml) gave a linear plot of log \(A/A\) against the square of the radius and yielded a molecular weight of 80,000 daltons.

Despite physical homogeneity, \(F_2\) (step 6) was found to have small amounts of other contaminating activities such as exonuclease and elongation factor \(G\).

1. Exonuclease assays showed that 1.6 \(\mu\)g of \(F_2\) released 0.5 m\(\mu\)mole of \(^{14}\)C-AMP from \(^{14}\)C-poly A in 15 minutes at 37\(^\circ\), which corresponds to a specific nuclease activity of 1.25 \(\mu\)moles/hr/mg protein. Assuming that the bulk of the contaminating nuclease is RNase II, it can be calculated from Singer's\(^9\) data for the specific activity of pure \(E. coli\) RNase II (2120 \(\mu\)moles/hr/mg protein, at 37\(^\circ\)) that RNase II contamination of \(F_2\) amounts to about 0.06 per cent.

2. Specific assay\(^8\) of \(F_2\) for \(G\) factor activity, as measured by poly U-dependent polyphenylalanine synthesis from Phe\(^{14}\)C\(\sim\)tRNA in the presence of excess T factor, gave a specific activity of 2.4 m\(\mu\)moles/10 min/mg, at 30\(^\circ\). The simultaneously measured specific activity of the purified \(G\) factor was 400. This represents a 0.6 per cent contamination. \(G\) factor activity in \(F_2\) was also determined by measuring the ribosome-stimulated GTPase activity. The specific activity was 0.09 \(\mu\)mole/10 min/mg, at 30\(^\circ\). If the specific GTPase activity of purified \(G\) factor is taken to be 20,\(^8\) contamination of \(F_2\) with \(G\) factor, as measured by this assay, is 0.45 per cent. This value is in good agreement with the previous one.

3. 1.9 \(\mu\)g of \(F_2\) gave no detectable T factor activity as assayed\(^8\) in the presence of excess \(G\).

\(F_2\) (step 6) is presumably free from initiation factor \(F_1\) and has no \(F_2\) activity. From the data given in this section, the purity of the preparation may be 99 per cent or higher.

**Relation of \(F_2\) to ribosomal proteins:** A comparison of gel electrophoretic patterns, at pH 4.5 (8.0 M urea), of \(F_2\) (step 6) and of the proteins from "unwashed" and 1.0 \(M\) NH\(_4\)Cl-washed 70\(S\) ribosomes is shown in Figure 3. Four of the more slowly moving components seen in the electropherogram of "unwashed" ribosomes (columns \(A(b)\) and \(C\)) are either missing (bands 1 and 4) or reduced in amount (bands 2 and 3) in the NH\(_4\)Cl-washed ribosomes (columns \(A(a)\) and \(D\)). The most acidic component (band 1) corresponds to \(F_2\) (cf. columns \(A(b)\), \(C\), and \(B\)). Gel electrophoresis at pH 4.5 of proteins derived from the NH\(_4\)Cl ribosomal wash shows the presence of bands 1 through 4 along with other more basic proteins.

**Effect of \(F_1\) and fraction \(S\) on the activity of \(F_2\):** Partially purified preparations of \(F_2\) (e.g., those described by Salas \textit{et al.})\(^{10}\) as assayed by AUG-dependent ribosomal binding of fMet\(\sim\)tRNA\(_f\), were always found to be active to a greater or lesser extent without the addition of other initiation factors. However, their activity was stimulated by \(F_1\) and, as reported in this paper, by fraction \(S\). It was therefore of interest to determine whether homogeneous \(F_2\) was still active in the absence of further factor additions. As shown in Table 2, \(F_2\) alone had 24 per cent of the activity obtained in the additional presence of \(F_1\) and fraction \(S\), and 37 or 33 per cent of the activity obtained upon the addition
Table 2. Effect of F₁ and fraction S on activity of F₂.*

<table>
<thead>
<tr>
<th>Factor additions</th>
<th>fMet(^{14}C)~tRNA bound (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) None</td>
<td>0.40</td>
</tr>
<tr>
<td>(b) F₂</td>
<td>1.40</td>
</tr>
<tr>
<td>(a) F₁</td>
<td>0.44</td>
</tr>
<tr>
<td>(b) F₂ + F₁</td>
<td>3.13</td>
</tr>
<tr>
<td>(a) S</td>
<td>0.25</td>
</tr>
<tr>
<td>(b) F₂ + S</td>
<td>3.28</td>
</tr>
<tr>
<td>(a) F₁ + S</td>
<td>0.27</td>
</tr>
<tr>
<td>(b) F₂ + F₁ + S</td>
<td>4.39</td>
</tr>
</tbody>
</table>

* Conditions of standard assay. The amounts of factors/sample were as follows: F₂ (step 6), 0.3 μg; F₁, 5.5 μg protein; and fraction S, 4.5 μg protein. Fraction S was a preparation obtained by ammonium sulfate fractionation and DEAE-cellulose chromatography.

of F₁ or fraction S, respectively. Whether the activity of step 6 F₂ by itself in the standard assay means that it can function in the absence of additional initiation factors, or whether this activity is due to contamination of the ribosomes with other factors, remains an open question at present.

We wish to thank Miss Roswitha Pagel for excellent technical assistance, Miss Mary-anne Pendergast for the photographs used in Figures 2 and 3, and Dr. Robert C. Warner and Mr. Frank Zaboretsky for the molecular weight determination. We are also indebted to Mr. Horace Lozina for growth of bacterial cells for preparation of ribosomes, and to Dr. Thomas H. Stoudt (Merck, Sharp, and Dohme, Rahway, N.J.) for generous supplies of large quantities of frozen *E. coli* Q13 cells for the preparation of initiation factors. The helpful contributions made by Miss Martha J. Miller, of this department, and Dr. Julian Gordon, Rockefeller University, have already been mentioned.

* Aided by grants AM-01845, FR-05099, and GM-01234 from the NIH, USPHS.
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