Chloroplast Leucyl-tRNA Synthetase from *Euglena gracilis*. Purification, Kinetic Analysis, and Structural Characterization†

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ABSTRACT: *Euglena gracilis* chloroplast leucyl-tRNA synthetase was purified to homogeneity by a series of steps including ammonium sulfate precipitation and chromatography on hydroxylapatite, DEAE-cellulose, Sepharose 6B, phosphocellulose, and Blue Dextran–Sepharose. The purified enzyme exhibits a specific activity of 1233 units/mg of protein, which is one of the highest specific activities obtained for an aminoacyl-tRNA synthetase prepared from plant cells. The enzyme has an apparent K_m value of 8×10^{-6} M for L-leucine, 1.3×10^{-4} M for ATP, and 1.3×10^{-6} M for $tRNA^{Leu}$. Chloroplast leucyl-tRNA synthetase appears to be a monomeric enzyme with a molecular weight of 100 000. The amino acid composition of chloroplast leucyl-tRNA synthetase has been determined. It is the first reported for a chloroplast aminoacyl-tRNA synthetase, and it reveals a relatively large proportion of apolar residues, as in the case of prokaryotic aminoacyl-tRNA synthetases.

In plant cells, chloroplasts possess their own protein synthesizing apparatus, including chloroplast-specific aminoacyl-tRNA synthetases (EC 6.1.1...). Previous studies have demonstrated that chloroplast aminoacyl-tRNA synthetases are different from their cytoplasmic counterparts, especially in their substrate (tRNA) specificity and in their behavior upon hydroxylapatite column chromatography (Reger et al., 1970; Krauspe & Parthier, 1974; Guillemaut & Weil, 1975; Guillemaut et al., 1975; Jeannin et al., 1976, 1978).

Up to now, only a few studies have been devoted to chloroplast aminoacyl-tRNA synthetases, but they have been performed on crude extracts or on partially purified enzymes and have essentially consisted in the determination of some catalytic properties (Burkard et al., 1970; Guderian et al., 1972; Krauspe & Parthier, 1974; Brantner & Dure, 1975). Structural characterization of a chloroplast aminoacyl-tRNA synthetase and comparison of its structural and catalytic properties with those of its cytoplasmic counterpart require the purification of the two enzymes from the green cells. But plant cells are very rich in proteases and phenolic compounds which may inactivate enzymes during the purification procedure (Loomis, 1974; Van Sumere et al., 1975; Rhodes, 1977; Wallace, 1977). This is probably the reason why very few chloroplast aminoacyl-tRNA synthetases have been purified and studied so far (Locy & Cherry, 1978; Imbault et al., 1979). As a first step in the comparative study of *Euglena gracilis* cytoplasmic and chloroplastic leucyl-tRNA synthetases, we have purified the chloroplast enzyme (EC 6.1.1.4) to homogeneity, as judged by the usual criteria of purity. We also present in this report some catalytic and structural properties of this enzyme, including its amino acid composition which is the first ever reported for a chloroplast aminoacyl-tRNA synthetase.

Experimental Procedures

Materials. DEAE-cellulose DE 52 and phosphocellulose P11 were obtained from Whatman and Sephadex G-50 and G-200, Sepharose 4B and 6B, and Blue Dextran 2000 from Pharmacia. ATP and bovine serum albumin were purchased from Sigma, L-[^{3}H]leucine (0.5 Ci/mM) and L-[^{14}C]leucine (20 μCi/μM) were from the "Commissariat à l'Energie Atomique" (Saclay, France), *Escherichia coli* tRNA was from Schwarz/Mann, yeast tRNA was from Boehringer, and Omnifluor was from New England Nuclear. All other chemicals were obtained from Merck.

Hydroxylapatite was prepared according to Siegelman et al. (1965) and Blue Dextran–Sepharose 4B according to Ryan & Vestling (1974).

Euglena gracilis Z cells were grown on the medium described by Cramer & Myers (1952) and modified by Padilla & James (1960), under continuous illumination at 25 °C, and harvested by centrifugation at 3500*g* at the end of the exponential growth phase. *Euglena* tRNAs were isolated as described by Meissner et al. (1971).

The following buffers used in the purification procedure: buffer A, 50 mM Tris-HCl[1] (pH 8), 1 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.1 mM EDTA, 0.002 mM L-leucine, 10% (v/v) propane-1,2-diol, and 10% (v/v) glycerol; buffer B, 50 mM potassium phosphate (pH 7.5), 1 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.1 mM EDTA, 0.002 mM L-leucine, and 15% (v/v) propane-1,2-diol; buffer B′, the same as buffer B but 400 mM in potassium phosphate (pH 6.5); buffer C, 50 mM potassium phosphate (pH 7), 1 mM $MgCl_2$, 0.002 mM L-leucine, 100 mM NaCl, and 15% (v/v) propane-1,2-diol; buffer D, 5 mM potassium phosphate (pH 6.5), 1 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.1 mM EDTA, 0.002 mM L-leucine, and 10% (v/v) glycerol; buffer D′, the same as buffer D but 300 mM in potassium phosphate (pH 7.5); buffer E, 10 mM Tris-HCl (pH 8), 3 mM $MgCl_2$, and 15% (v/v) propane-1,2-diol; buffer F, 10 mM Tris-HCl (pH 8), 4 mM $MgCl_2$, 3 mM ATP (neutralized with NaOH), 0.002 mM L-leucine, and 15% (v/v) propane-1,2-diol.

Methods. Aminoacylation Reaction. Chloroplast leucyl-tRNA synthetase (LeuRS)[1] activity was determined by measuring the aminoacylation of *Euglena* or *E. coli* tRNA in a 100-μL incubation mixture containing 20 mM Tris-HCl (pH 8), 5 mM β-mercaptoethanol, 7.5 mM ATP, 7.5 mM magnesium acetate, 0.1 mg/mL bovine serum albumin, 0.008 mM L-[^{3}H]leucine (0.5 Ci/mmol) when the column fractions are tested or 0.06 mM L-[^{14}C]leucine (20 μCi/μmol) when

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[1] Abbreviations used: LeuRS, leucyl-tRNA synthetase; ValRS, valyl-tRNA synthetase; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

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Table I: Purification of *Euglena* Chloroplast LeuRS

stage	proteins (mg)	sp act. (units/ mg of protein)	units (no.)	purification (fold)	yield (%)
supernatant 35000*g*	9100				
ammonium sulfate precipitation	7400				
hydroxylapatite chromatography	4170	2.3	9591	1	100
DEAE-cellulose chromatography	1462	4	5850	1.7	61
ammonium sulfate precipitation	559	6	3357	2.6	35
Sepharose 6B filtration	105	21	2205	9.1	23
phosphocellulose chromatography	27	55	1485	23.9	15
Blue Dextran–Sepharose chromatography	0.56	1233	690	536	7

the specific activity of the enzyme is measured, 0.9 mg/mL tRNA, and various amounts of enzyme. After incubation at 30 °C for 5 min (when column fractions are tested) or for various time intervals (when enzyme specific activity is measured) a 80-μL aliquot was put on a Whatman 3MM paper disk, which was washed according to Mans & Novelli (1961), dried, and put into 5 mL of Omnifluor solution (4 g/L of toluene). The radioactivity of the aminoacyl-tRNA retained on the disk was measured in a liquid scintillation counter (Intertechnique).

One unit of enzyme is defined as the amount of enzyme which catalyzes the attachment of 1 nmol of leucine to tRNA in 1 min at 30 °C under the above described conditions.

Kinetic Measurements. All substrates, except one whose concentration was variable, were present in saturating concentrations. Reactions were started by adding ATP or tRNA solutions previously equilibrated at 30 °C. The amounts of [^{14}C]leucyl-tRNA formed during at least eight different incubation times were used to determined the initial velocity of the reaction. For apparent K_m determinations, the kinetic data were analyzed according to Lineweaver & Burk (1934).

Determination of Protein Concentration. Protein concentration was determined in column effluents according to Ehresmann et al. (1973) by using bovine serum albumin as a standard and in pure enzyme solutions by their optical density at 280 nm using an extinction coefficient $\epsilon^{1\ mg/mL}_{280nm}$, of 0.97, as calculated from the amino acid composition.

Polyacrylamide Gel Electrophoresis. The purified enzyme was denatured by incubation at 100 °C for 3 min in the presence of 1% (w/v) $NaDodSO_4$, 4 M urea, and 10 mM dithiothreitol. Electrophoresis was performed according to Laemmli (1970) on a 7.5% polyacrylamide gel containing 0.1% (w/v) $NaDodSO_4$ and 4 M urea. After the electrophoresis, the proteins were stained with Coomassie Brilliant Blue.

Amino Acid Analysis. Samples were thoroughly dialyzed against 0.5% (v/v) acetic acid, freeze-dried, and hydrolyzed in a sealed tube under nitrogen in 6 N HCl at 110 °C for 24 h, with a crystal of phenol to protect tyrosine from oxidation. Analyses were performed on a Durrum D500 amino acid analyzer. Cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively, after hydrolysis of the performic-oxidized enzyme according to Moore (1963). Tryptophan was determined after hydrolysis by methanesulfonic acid was described by Liu & Chang (1971).

Results

Purification of Euglena Chloroplast LeuRS. The whole purification procedure was performed at 4 °C. Freshly harvested *Euglena* cells (300 g) were suspended in 300 mL of buffer A made 2% (w/v) in poly(ethylene glycol) 6000 and disrupted in a French press (Aminco) at 12 000 psi. The homogenate was centrifuged at 35000*g* for 30 min, and ammonium sulfate was added to 70% saturation. The resulting

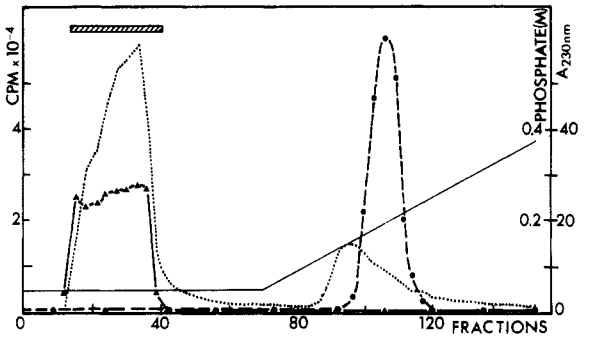

FIGURE 1: Hydroxylapatitie column activity. Chloroplast LeuRS activity was measured by using *E. coli* tRNA (▲) and cytoplasmic LeuRS activity by using yeast tRNA (●); (···) A_{230nm}; (—) potassium phosphate concentration; (slashed bar) fractions collected and pooled for the next purification step.

precipitate was redissolved in buffer B and desalted on a Sephadex G-50 column (7 × 40 cm) equilibrated with the same buffer, and the fractions containing the proteins (as monitored by absorption at 230 nm) were applied to a hydroxylapatite column (6 × 19 cm) equilibrated with buffer B. Under these conditions (potassium phosphate, 50 mM), the chloroplast LeuRS was not retained on the column as shown in Figure 1 (the fact that the activity peak is truncated indicates that tRNA was probably limiting in the assay mixture). Fractions containing chloroplast LeuRS activity were applied directly to a DEAE-cellulose column (5 × 14 cm) equilibrated with buffer B. After the column was washed with the same buffer, the enzyme was eluted by a linear gradient of molarity and pH using buffers B (1 L) and B′ (1 L). The fractions containing chloroplast LeuRS (as measured by the aminoacylation of *E. coli* tRNA) were pooled, and ammonium sulfate was added to 70% saturation. The precipitate was dissolved in buffer C and passed through a Sepharose 6B column (2.5 × 130 cm) equilibrated with buffer C. Active fractions were pooled, extensively dialyzed against buffer D, and loaded on a phosphocellulose column (5 × 25 cm) equilibrated with buffer D. After the column was washed with the same buffer, elution was performed with a linear gradient of molarity and pH using buffers D (1 L) and D′ (1 L). Fractions containing the chloroplast LeuRS (as measured by aminoacylation of *E. coli* tRNA) were pooled, dialyzed against buffer E, and applied to a Blue Dextran–Sepharose column (5 × 20 cm) equilibrated with buffer E. After the column was washed with buffer E, elution of the chloroplast LeuRS was performed with ATP and L-leucine in buffer F. At this step, the purified enzyme was concentrated in a final volume of 2 mL by filtration through a Diaflo PM 10 membrane under nitrogen pressure.

Table I summarizes the purification procedure of chloroplast LeuRS. Before the hydroxylapatite step, *Euglena* chloroplast and cytoplasmic LeuRS are not separated, and since they are both able to aminoacylate *E. coli* tRNA, is not possible to

Table II: Amino Acid Composition of *Euglena* Chloroplast LeuRS and Comparison with LeuRS from *E. coli* and *B. stearothermophilus*

amino acid	*Euglena* chloroplast LeuRS (residues/ molecule)	*E. coli*[a] LeuRS (residues/ molecule)	*B. stearothermophilus*[b] LeuRS (residues/ molecule)
Lys	46	56	65
His	19	17	26
Arg	50	42	47
Trp	10[c]	24[d]	24[d]
Asx	75	93	93
Thr	46	54	50
Ser	37	26	33
Glx	121	95	120
Pro	60	41	30
Gly	84	61	72
Ala	89	78	67
$^1/_2$-Cys[e]	17	6	6
Val	62	72	64
Met[f]	16	30	33
Ile	34	37	51
Leu	83	64	77
Tyr	31	33	29
Phe	31	29	34
M_r	100 000	105 000	110 000

[a] From Hayashi et al. (1970). [b] From Koch et al. (1974). [c] Determined after methanesulfonic acid hydrolysis as described by Liu & Chang (1971). [d] Estimated by the method of Edelhoch (1967). [e] Determined as cysteic acid. [f] Determined as methionine sulfone.

distinguish them to estimate their activity and to determine the degree of purification. *Euglena* chloroplast LeuRS exhibits one of the highest specific activities (1233 units/mg of protein) obtained so far for a plant aminoacyl-tRNA synthetase.

Control of Enzyme Purity. No other aminoacyl-tRNA synthetase activity could be detected when aminoacylation tests were performed with 15 other amino acids.

Polyacrylamide gel electrophoresis of the purified chloroplast LeuRS in the presence of $NaDodSO_4$ and urea yields a single protein band (Figure 2), as revealed by staining with Coomassie Brilliant Blue. When the gel is stained with periodic acid–Schiff reagent, according to Zacharius et al. (1969), a band appears, which coincides with protein band. This result raises the possibility that *Euglena* chloroplast LeuRS is a glycoprotein.

Molecular Weight. A molecular weight of 100 000 was found for the denatured chloroplast LeuRS upon polyacrylamide gel electrophoresis in urea–$NaDodSO_4$. Gels were calibrated by using RNA polymerase (α subunit M_r 39 000; β and β' subunits M_r 160 000, yeast ValRS (M_r 125 000), bovine serum albumin (M_r 68 000), and soybean trypsin inhibitor (M_r 21 000) as markers.

A similar value of M_r 100 000 was found for the nondenatured chloroplast LeuRS, using molecular filtration on a Sephadex G-200 column (1 × 42 cm) calibrated with catalase

FIGURE 2: Polyacrylamide gel electrophoresis of the purified chloroplast LeuRS under denaturating conditions. A 30-μg sample of purified enzyme was denatured as described under Experimental Procedures and submitted to electrophoresis under 1.5 mA on a 7.5% polyacrylamide gel containing urea and $NaDodSO_4$.

(M_r 240 000), alcohol dehydrogenase (Mr 150 000), yeast ValRS (M_r 125 000), and bovine serum albumin (M_r 68 000).

The fact that denatured and nondenatured *Euglena* chloroplast LeuRS exhibit the same molecular weight suggests a monomeric structure for the enzyme.

Amino Acid Composition. The amino acid composition of *Euglena* chloroplast LeuRS is reported in Table II and compared to those of LeuRS from *E. coli* (Hayashi et al., 1970) and *B. stearothermophilus* (Koch et al., 1974). The results are the mean of two complete analyses performed on two different enzyme preparations. The contribution of some groups of residues to the total amino acid composition of chloroplast LeuRS was compared to the average values calculated by Dayhoff et al. (1978) from 314 known proteins (Table III). Moreover, the ratio of hydrophilic over apolar amino acids was estimated according to Hatch (1965). The value of this ratio was found to be lower for chloroplast LeuRS (1.31), *E. coli* LeuRS (1.33), and *B. stearothermophilus* LeuRS (1.40) than that of most proteins as calculated by Hatch (1965).

Catalytic Properties. The aminoacylation reaction catalyzed by chloroplast LeuRS has an optimal pH of 8.0 in 20 mM Tris-HCl buffer. The optimal value for the Mg^{2+}/ATP ratio was found to be 1.2. Low KCl concentrations are unable to stimulate chloroplast LeuRS activity, but concentrations greater than 30 mM inhibit the enzyme. Mg^{2+} ions can be partially replaced by other divalent cations such as Ca^{2+} (80% of the maximum activity obtained with Mg^{2+}), Mn^{2+} (75%), and CO_2^+ (40%).

Table III: Contribution of Some Groups of Amino Acids to the Total Composition (mol %)

group	*Euglena* chloroplast LeuRS	*E. coli*[a] LeuRS	*B. stearothermophilus*[b] LeuRS	average values[c] of proteins
acidic + acid amide: Asx + Glx	21.5	21.9	23.1	19.8
basic: Lys + Arg + His	12.6	13.4	15.0	13.5
aromatic: Tyr + Phe + Trp	7.9	10.0	9.4	8.3
hydroxyl: Ser + Thr	9.1	9.3	9.0	13.1
hydrophobic: Leu + Ile + Val + Met	21.4	23.7	24.4	20.2

[a] From Hayashi et al. (1970). [b] From Koch et al. (1974). [c] From Dayhoff et al. (1978).

Table IV: Apparent K_m Values of Chloroplast LeuRS for Various Substrates

substrate	K_m (M)
L-leucine	8.0×10^{-6}
ATP	1.3×10^{-4}
Euglena gracilis $tRNA^{Leu}$ [a]	1.3×10^{-6}
E. coli $tRNA^{Leu}$ [a]	8.8×10^{-7}

[a] Apparent K_m values were determined by using total tRNA. The percentage of $tRNA^{Leu}$ contained in each total tRNA preparation was deduced from the plateau of aminoacylation of total tRNA with leucine.

The apparent K_m values of chloroplast LeuRS have been determined with respect to the aminoacylation reaction and are presented in Table IV. The fact that apparent K_m values for *Euglena* $tRNA^{Leu}$ and for *E. coli* $tRNA^{Leu}$ are very similar justifies the use of commercially available *E. coli* tRNA (instead of *Euglena* tRNA) when column fractions are tested.

Discussion

In order to protect enzymes from degradation by proteases and phenolic compounds present in plant cells, it is essential to include protective agents in the buffers used and to go through the purification procedure as quickly as possible. EDTA and poly(ethylene glycol) 6000 were found to be very effective enzyme protectors against proteases and phenolic compounds, respectively. The presence of a mixture of 10% (v/v) glycerol and 10% (v/v) propane-1,2-diol in the homogenization buffer A was found to allow a better stabilization of chloroplast LeuRS than the presence of either agent alone, even when its concentration is increased. The addition of 15% (v/v) propane-1,2-diol in other buffers provides as good a stabilization of chloroplast LeuRS as the addition of 50% (v/v) glycerol, with the advantage of a much lower viscosity.

Chromatography on hydroxylapatite columns has been shown by Krauspe & Parthier (1974) to be a good method to separate chloroplast and cytoplasmic aminoacyl-tRNA synthetases from *Euglena*. Under the conditions used in our studies (50 mM potassium phosphate, pH 7.5), the chloroplast LeuRS is not retained on hydroxylapatite (Figure 1) and can be directly applied to the DEAE-cellulose chromatographic column. Cytoplasmic LeuRS is retained on hydroxylapatite and can be eluted by a linear phosphate gradient.

DEAE-cellulose chromatography and ammonium sulfate precipitation provide only a 2.6-fold purification with a loss of 65% of the activity, but these two steps appear necessary to eliminate some contaminating proteins which are not removed by the other purification steps.

The most efficient step of our chloroplast LeuRS purification procedure is affinity chromatography on the Blue Dextran–Sepharose column. ATP and amino acid have been used to elute chloroplast LeuRS from the column. The cognate tRNA would probably be a more specific eluant, but unfortunately no $tRNA^{Leu}$ isoacceptor is at present available in sufficient amounts.

The fact that chloroplast LeuRS was found to be a M_r 100 000 monomer is in good agreement with results concerning other organisms for which LeuRS has been described as a large monomer: *E. coli*, M_r 104 000 (Rouget & Chapeville, 1970); *Bacillus stearothermophilus*, M_r 110 000 (Koch et al., 1974); *Candida utilis*, M_r 128 000 (Murasugi & Hayashi, 1975); *Tetrahymena pyriformis* (cytoplasmic and mitochondrial), M_r 110 000 (Chiu & Suyama, 1975); Baker's yeast, M_r 115 000 (Lin et al., 1979) or M_r 120 000 (Kern et al., 1981).

The data obtained with purified chloroplast LeuRS provide the first information on the structure of a chloroplast aminoacyl-tRNA synthetase and allow a comparison with well-characterized prokaryotic LeuRS from *E. coli* (Hayashi et al., 1970) and *B. stearothermophilus* (Koch et al., 1974). All three enzymes have in common a low content of hydroxyl groups as compared to the average value for proteins. Moreover, the relatively low value of the ratio of polar to apolar amino acid residues (1.31) suggests that chloroplast LeuRS has a rather compact structure stabilized by hydrophobic interactions.

The comparison of the structural and catalytic properties of *Euglena* chloroplast LeuRS and of its cytoplasmic counterpart should provide interesting information about the similarities between these two enzymes which catalyze the same reaction in two different compartments of the plant cell.

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Fluorescence Polarization Studies of the Interaction of *Escherichia coli* Protein Synthesis Initiation Factor 3 with 30S Ribosomal Subunits†

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ABSTRACT: Steady-state fluorescence polarization techniques were used to study the binding of initiation factor 3 (IF3) to 30S ribosomal subunits. Covalent fluorescent derivatives of IF3 were prepared by treating the pure protein with fluorescein isothiocyanate. The fluorescein-labeled IF3 (F-IF3) contained 0.8–1.7 dye molecules per protein. Polyacrylamide gel electrophoretic analysis of the derivatized forms is consistent with the view that the probe is randomly attached, presumably to lysine ϵ-amino groups. The activity of F-IF3 is not impaired in assays for binding to 30S ribosomal subunits or in promoting formylmethionyl-tRNA binding to 70S ribosomes. Fluorescence polarization values were measured at different F-IF3 and 30S ribosomal subunit concentrations, and the association constant and number of binding sites were calculated. In buffer containing 10 mM magnesium acetate and 100 mM ammonium chloride, the association constant is (3.1 ± 1.4) × 10^7 M^{-1}, and the number of ribosomal binding sites is 1.2 ± 0.2. The value for the association constant varies inversely with the ammonium chloride and magnesium acetate concenrations by a small amount. Competition studies show that nonderivatized IF3 binds to 30S ribosomal subunits with the same affinity as F-IF3. Therefore, the association constants measured for F-IF3 are valid for IF3 as well.

Initiation of protein synthesis in bacteria involves the sequential formation of 30S and 70S initiation complexes containing mRNA and fMet-$tRNA_f$.[1] In *Escherichia coli*, three initiation factors promote this process: IF1 (M_r 8119); IF2 (M_r 115 000); and IF3 (M_r 20 668). A number of pathways have been proposed to describe the interaction of the macromolecular components [for reviews, see Grunberg-Manago et al. (1978) and Lodish (1976)], but numerous ambiguities or uncertainties exist, and no single pathway has been rigorously proven to be correct. We lack the kinetic data which could be used to distinguish reasonable alternative pathways.

In this work, we utilize the technique of steady-state fluorescence polarization to measure the equilibrium association constant for fluorescein-labeled IF3 (F-IF3) binding to the 30S ribosomal subunit. Changes in rotational mobility are reflected in changes in the polarization of fluorescence emission. Large differences in the rotational mobility of free initiation factors compared to those complexed with ribosomal particles make this technique attractive for the study of factor–ribosome interactions. Theoretical treatments of fluorescence polarization were given originally by Perrin (1926) and more recently by Weber (1952, 1971). Steady-state methods were first used in studies of dye–protein (Laurence, 1952), protein–protein, and protein–nucleic acid associations (Steiner, 1953). Further important contributions in the theoretical and experimental uses of fluorescence polarization resulted from work on the equilibria (Dandliker & Feigen, 1961; Dandliker et al., 1964, 1973; Kierszenbaum et al., 1969; Portmann et al., 1975) and rate kinetics (Dandliker & Levison, 1967; Levison et al., 1970, 1971, 1975; Levison, 1975) of antigen–antibody reactions. We demonstrate here and elsewhere[2] that the steady-state fluorescence polarization technique is suitable for studying the binding of factors to ribosomes. A preliminary account of our studies with fluorescein-labeled

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[1] Abbreviations used: fMet-tRNA, formylmethionyl transfer RNA; IF, initiation factor; F-IF3, fluorescein-labeled IF3; βME, 2-mercaptoethanol; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

[2] J. Weiel and J. W. B. Hershey, unpublished experiments.

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