Characterization of Initiation Factor 3 from Wheat Germ. 1. Effects of Proteolysis on Activity and Subunit Composition†

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ABSTRACT: Wheat germ initiation factor 3 (eIF-3) is a large (15 S) particle containing 10 subunits with molecular weights ranging from 28 000 to 116 000. Two forms of wheat germ eIF-3 which differ in ability to support polypeptide synthesis in vitro have been obtained by chromatography on carboxymethyl-Sephadex (CM-Sephadex). The less active form is not retained on CM-Sephadex in 50 mM KCl and contains lower amounts of two subunits, the 116 000-dalton polypeptide (pp116) and the 36 000-dalton polypeptide (pp36). The more active form is retained on CM-Sephadex in 50 mM KCl and is eluted by 150 mM KCl. Treatment of the more active form with small amounts of trypsin results in a rapid degradation of four of the subunits (pp116, pp107, pp87, and pp36) and in a rapid loss in the ability to support polypeptide synthesis. Trypsin treatment also diminishes the ability of eIF-3 to support the binding of mRNA to 40S ribosomal subunits. These findings indicate that pp116, pp107, pp87, and pp36 are in exposed positions in the eIF-3 particle and that pp116 and/or pp36 are essential for activity.

Initiation factor 3 (eIF-3)[1] has been isolated from a number of eukaryotic sources, including rabbit reticulocytes (Safer et al., 1976; Benne & Hershey, 1976; Trachsel et al., 1977), HeLa cells (Brown-Luedi et al., 1982), rat liver (Nygard & Westermann, 1982a), and wheat germ (Spremulli et al., 1979; Ceglarz et al., 1980; Checkley et al., 1981; Seal et al., 1983), and in all cases has been shown to be a large particle (15 S) containing at least 10 subunits. eIF-3 binds to 40S ribosomal subunits, stabilizes the binding of Met-tRNA$_i$, and is essential for the binding of mRNA to 40S ribosomal subunits (Trachsel et al., 1977; Benne & Hershey, 1978; Trachsel & Staehelin, 1979; Erni & Staehelin, 1983). Nygard & Westermann (1982b) showed that the 66 000-dalton polypeptide of rat liver eIF-3 can be cross-linked to 18S ribosomal RNA. Except for this report, little is known about the function of the various subunits of eIF-3 or their physical arrangement in the intact particle.

Here we report the effects of proteolysis on the activity and subunit composition of wheat germ eIF-3. The results obtained indicate that four of the subunits of eIF-3 (pp116, pp107,pp87, and pp36) are susceptible to proteolytic degradation, strongly suggesting that these polypeptides are in exposed positions in the eIF-3 particle. The data also indicate that pp116 and/or pp36 are essential for activity.

Materials and Methods

Materials. Wheat germ was generously supplied by J. M. deRosier (International Multifoods, Minneapolis, MN) and stored at −20 °C. The 0–40% and 40–60% ammonium sulfate fractions of the 120 mM KCl postribosomal supernatant, salt-washed wheat germ ribosomes, 40S ribosomal subunits, yeast polysomal RNA, [^{35}S]Met-tRNA$_i$, and eIF-4B (fraction 2A) were prepared as previously described (Spremulli et al., 1977, 1979). eIF-2 and eIF-4A were prepared by modifications of previously described procedures (Lax et al., 1982). STNV RNA was purified as described by Clark & Klein (1974) and iodinated by a modification of the procedure of Commerford (1971). Glutaraldehyde was from Polysciences, Inc. [^{14}C]Leucine and [γ-^{32}P]ATP were from New England Nuclear. TPCK-treated trypsin was from Worthington, and soybean trypsin inhibitor was from Sigma.

Assays. (*A*) *eIF-3-Dependent Polypeptide Synthesis.* The reaction mixture contained the following in 100 μL: 24 mM Hepes/KOH, pH 7.6, 2.4 mM DTT, 0.1 mM spermine, 3 mM $Mg(OAc)_2$, 40 mM KCl, 100 mM KOAc, 34 μM [^{14}C]-leucine, 50 μM each of the other 19 amino acids, 1 mM ATP, 0.2 mM GTP, 7.8 mM creatine phosphate, 3 μg of creatine kinase, 0.75 A_{260} unit of yeast polysomal RNA, 500–600 μg of the 40–60% ammonium sulfate fraction of the 120 mM KCl postribosomal supernatant, 4.6 μg of eIF-4B (fraction 2A), and eIF-3 as indicated. After incubation at 25 °C for 30 min, the amount of [^{14}C]leucine incorporated into hot trichloroacetic acid insoluble material was determined as previously described (Walthall et al., 1979) except that glass fiber filters were used in place of nitrocellulose filters. A unit of eIF-3 activity is defined as 1 pmol of leucine incorporated into polypeptide under these conditions.

(*B*) *Binding of eIF-3 to 40S Ribosomal Subunits.* The reaction mixture contained the following in 100 μL: 24 mM Hepes/KOH, pH 7.6, 2.4 mM DTT, 0.1 mM spermine, 4 mM $Mg(OAc)_2$, 50 mM KCl, 0.12 A_{260} unit (8 pmol) of 40S ribosomal subunits, and ^{32}P-eIF-3 as indicated. After incubation at 25 °C for 10 min, 10 μL of 5.5% glutaraldehyde was added, and an aliquot (90 μL) was layered on top of a 5–30%

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[1] Abbreviations: eIF-3, initiation factor 3; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; PMSF, phenylmethanesulfonyl fluoride; STI, soybean trypsin inhibitor; EDTA, ethylenediaminetetraacetic acid; CM, carboxymethyl; STNV, satellite tobacco necrosis virus; SDS, sodium dodecyl sulfate; GMP-PNP, 5′-guanylyl imidodiphosphate.

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sucrose density gradient and treated as described below.

(C) eIF-3-Dependent Binding of mRNA to 40S Ribosomal Subunits. The reaction mixture contained the following in 100 μL: 24 mM Hepes/KOH, pH 7.6, 2.4 mM DTT, 0.1 mM spermine, 4 mM $Mg(OAc)_2$, 50 mM KCl, 1 mM ATP, 0.2 mM GMP-PNP, 7.8 mM creatine phosphate, 3 μg of creatine kinase, 18 pmol of [^{35}S]Met-tRNA (<50 cpm/pmol), 0.12 A_{260} unit (8 pmol) of 40S ribosomal subunits, 1.6 μg of eIF-2, 4.5 μg of eIF-4A, 10 μg of eIF-4B, 3 μg of ^{125}I-labeled STNV RNA (5000–9000 cpm/pmol assuming M_r 400 000), and eIF-3 as indicated. After incubation at 25 °C for 10 min, a 90-μL aliquot was layered on top of a 5–30% sucrose density gradient and treated as described below.

Sucrose Density Gradient Analysis. Five-milliliter, 5–30% linear sucrose gradients were prepared in buffer containing 20 mM Hepes/KOH, pH 7.6, 4 mM $Mg(OAc)_2$, 6 mM 2-mercaptoethanol, and 50 mM KCl. Samples (90 μL) were layered on the top, and the tubes were centrifuged for 2 h at 48 000 rpm in a Beckman SW 50.1 rotor. Fractions of 0.4 mL were collected, diluted with 1 mL of buffer W [10 mM Hepes/KOH, pH 7.6, 4 mM $Mg(OAc)_2$, and 50 mM KCl], and passed over a nitrocellulose filter (Schleicher & Schuell, 0.45 μm). The filter was washed twice with 2 mL of buffer W, and the amount of radioactivity retained by the filter was measured in a liquid scintillation counter.

Purification of eIF-3. Wheat germ eIF-3 was purified by a modification of the procedure of Checkley et al. (1981). The phosphocellulose column was developed with a stepwise KCl gradient rather than a linear gradient. The column was washed with buffer B (20 mM Hepes/KOH, pH 7.6, 1 mM DTT, 0.1 mM EDTA, and 10% glycerol) containing 150 mM KCl (buffer B-150) followed by buffer B-300. eIF-3 eluted from the column in 300 mM KCl. The active fractions were pooled and dialyzed against buffer B-50, and the sample (6 mL containing 16 mg of protein) was applied to an 8-mL CM-Sephadex column equilibrated in buffer B-50. After the column was washed with buffer B-50, eIF-3 was eluted from the column with buffer B-150. The active fractions were pooled, divided into small aliquots, and stored at −70 °C. Protein concentrations were determined by the method of Bradford (1976) with bovine serum albumin as a standard.

SDS–Polyacrylamide Gel Electrophoresis. SDS–polyacrylamide gel electrophoresis was carried out in a 12.5% polyacrylamide slab gel according to the procedure of Laemmli (1970) as modified by Staehelin et al. (1979). The gel was stained with Coomassie Brilliant Blue R and then destained. The intensities of the polypeptide bands were determined by scanning the gel with a densitometer (E-C Apparatus, Inc.) and measuring the area under each peak with a planimeter. The molar ratios were determined according to the method of Checkley et al. (1981) except that values were normalized to pp56. Polypeptide 56 did not appear to vary from preparation to preparation and was not degraded by trypsin.

Trypsin Treatment of eIF-3. eIF-3, 465 μg of the CM-150 fraction in 750 μL of buffer B-150, and TPCK-treated trypsin, 1 μg/mL in buffer B-100, were incubated separately at 25 °C for 1 min. A 150-μL aliquot of eIF-3 was removed and placed on ice to be used as a control. To the remainder of the eIF-3 (372 μg in 600 μL) was added 93 μL of the trypsin solution (ratio of trypsin/eIF-3 = 1/4000). The mixture was incubated at 25 °C, and aliquots of 150 μL were removed at the times indicated. Twenty microliters of a 10 μg/mL solution of STI in buffer B-100 was added to stop proteolysis, and the samples were placed in ice. A portion of the sample was used to determine eIF-3 activity in the in vitro polypeptide synthesis assay, and a portion was analyzed by SDS–polyacrylamide gel electrophoresis.

Table I: Purification of Wheat Germ eIF-3 by Column Chromatography on CM-Sephadex

step	protein (mg)	units (×10^{-3})	sp act.
phosphocellulose[a]	16	335	21 000
CM-Sephadex			
50 mM KCl	6.5	40	6 150
150 mM KCl	4.7	165	35 000

[a] Obtained as described under Materials and Methods.

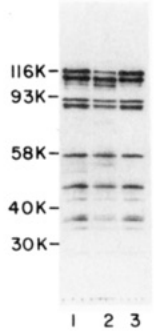

FIGURE 1: Analysis of the PC, CM-50, and CM-150 fractions by SDS–polyacrylamide gel electrophoresis. Samples of eIF-3 were electrophoresed in a 12.5% SDS–polyacrylamide slab gel as described under Materials and Methods. Lane 1, PC fraction, 12 μg; lane 2, CM-50 fraction, 12 μg; lane 3, CM-150 fraction, 10 μg.

Phosphorylation of eIF-3. eIF-3 was phosphorylated by a wheat germ kinase (Yan & Tao, 1982) as previously described (Browning et al., 1985). The phosphorylation reaction mixture contained the following in 400 μL: 24 mM Hepes/KOH, pH 7.6, 1 mM DTT, 140 mM KCl, 1 mM $Mg(OAc)_2$, 30 μM [γ-^{32}P]ATP (2700 cpm/pmol), 10 μg of wheat germ kinase, and 375 μg of eIF-3. This mixture was incubated at 25 °C for 15 min and then placed on ice. ^{32}P-eIF-3 was separated from free [γ-^{32}P]ATP by centrifugation through a 1-mL Sephadex G-50 column as described by Penefsky (1977) and was stored at −70 °C.

RESULTS

eIF-3 was purified from wheat germ by fractional ammonium sulfate precipitation followed by chromatography on DEAE-cellulose and phosphocellulose. Attempts to purify the factor further by either glycerol gradient centrifugation or molecular sieve chromatography on Sephadex G-200 did not result in a significant purification of the factor. Thus, a new purification method, chromatography on CM-Sephadex, was employed. eIF-3 that had been purified through the phosphocellulose step (PC fraction) was applied to a CM-Sephadex column equilibrated in buffer containing 50 mM KCl. As shown in Table I, 10–20% of the eIF-3 activity applied to the column was found in the 50 mM KCl wash (fraction CM-50). Approximately 50% of the eIF-3 activity was retained on the column and eluted in 150 mM KCl (fraction CM-150). The CM-150 fraction had a specific activity 1.5–2 times greater than the input material and 5–6 times greater than the CM-50 fraction. When the CM-50 fraction was applied to a second CM-Sephadex column equilibrated in 50 mM KCl, little, if any, eIF-3 activity was retained by the column.

Analysis of the PC fraction, CM-50 fraction, and CM-150 fraction by SDS–polyacrylamide gel electrophoresis (Figure 1) showed that all 3 fractions contained the same 10 major polypeptides. The CM-50 fraction, however, contained significantly smaller amounts of two polypeptides, pp116 and pp36. The molar ratios of the various subunits were calculated

Table II: Approximate Molecular Weights and Molar Ratios of Polypeptides in Fractions CM-50 and CM-150

polypeptide	M_r ($\times 10^{-3}$)[a]	molar ratio[b] CM-50 fraction	molar ratio[b] CM-150 fraction
1	116	0.3	1.0
2	107	0.9	1.0
3	87	0.5	0.5
4	83	0.7	0.8
5	56	1.0[c]	1.0[c]
6	45	1.1	1.1
7	41	0.5	0.5
8	36	1.2	1.7
9	34	0.6	0.6
10	28	0.4	0.4

[a] Determined by SDS–polyacrylamide slab gel electrophoresis. [b] Determined as described under Materials and Methods. [c] Arbitrarily given a value 1.0.

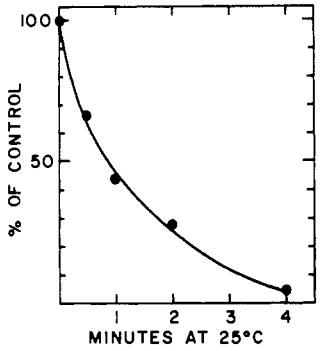

FIGURE 2: Effect of trypsin on the activity of eIF-3. eIF-3 (CM-150 fraction) was treated with trypsin as described under Materials and Methods for the times indicated. Aliquots containing 0.7 μg of eIF-3 were assayed in duplicate for the ability to support in vitro polypeptide synthesis. The untreated control supported the incorporation of 25 pmol of [^{14}C]leucine into polypeptide.

and are shown in Table II. The amount of pp116 in the CM-50 fractions was reduced 70%, and the amount of pp36 was reduced 30%. The amounts of pp107 and pp83 were reduced about 10%, and the remainder of the polypeptides did not appear to be reduced significantly. These data indicate that pp116 and pp36 are degraded by endogenous proteases in vivo or during the isolation procedure, despite the addition of PMSF and STI to the extraction buffer. The data also indicate that pp116 and/or pp36 are essential for activity.

To determine which of the subunits of eIF-3 are most sensitive to proteolysis and which of the subunits are essential for activity, eIF-3 (fraction CM-150) was treated with low levels of trypsin. As shown in Figure 2, the ability of eIF-3 to support in vitro polypeptide synthesis was reduced by 50% after incubation with trypsin for 1 min, and activity was reduced by over 85% after 4 min. To determine the effect of trypsin treatment on the subunit composition of eIF-3, the trypsin-treated samples were analyzed by SDS–polyacrylamide gel electrophoresis. As shown in Figure 3, very little degradation of six of the subunits (pp83, pp56, pp41, pp34, and pp28) was observed after 8 min of incubation with trypsin, indicating that either these polypeptides contain very few arginine or lysine residues or these amino acids are not accessible to the trypsin. The four other subunits of eIF-3, pp116, pp107, pp87, and pp36, were degraded by treatment with trypsin. Polypeptide 116 was degraded the most rapidly; it was reduced to 50% of the control after 2 min and to approximately 15% of the control after 8 min. The degradation of pp87 was also quite rapid, although somewhat slower than that of pp116. After 8 min of treatment, only 25% of this

Table III: Effect of Trypsin Treatment on the Ability of eIF-3 To Bind to 40S Ribosomal Subunits[a]

eIF-3 added (pmol)	^{32}P-eIF-3 bound (pmol)
untreated	
12	2.2
24	4.1
treated	
12	1.8
24	3.7

[a] eIF-3 and eIF-3 treated with trypsin for 1 min were phosphorylated as described under Materials and Methods. Untreated eIF-3 contained 4400 cpm/pmol (assuming a molecular weight of 700 000 for eIF-3), and trypsin-treated eIF-3 contained 5600 cpm/pmol. Assay conditions and sucrose density gradient analysis were as described under Materials and Methods.

Table IV: Effect of Trypsin Treatment on the Ability of eIF-3 To Support the Binding of mRNA to 40S Ribosomal Subunits[a]

eIF-3 added	^{125}I-labeled STVN RNA bound (pmol)	% control
untreated, 8 pmol	0.76	100
trypsin treated, 8 pmol	0.37	49
none	<0.05	<7

[a] Binding of ^{125}I-labeled STNV RNA to 40S ribosomal subunits and treatment of eIF-3 with trypsin for 1 min were as described under Materials and Methods. ^{125}I-Labeled STNV RNA contained 9000 cpm/pmol, assuming a molecular weight of 400 000 for STNV RNA.

polypeptide remained. Polypeptides 107 and 36 were degraded more slowly; approximately 50% of these polypeptides remained after 4 min. Continuing the incubation for 8 min reduced the amount of these polypeptides to 40% of the control. However, the degradation of pp36 can be viewed in another manner. Polypeptide 36 is the only subunit present in close to a 2/1 molar ratio. It is not known at this time whether this is due to the presence of two identical polypeptides or two nonidentical polypeptides with approximately the same molecular weight. If it is assumed that the latter view is correct and that proteolysis of only one of the two polypeptides occurs, then a different assessment can be made. This is represented in Figure 3 by the curve designated pp36* which indicates that one of the 36 000-dalton polypeptides was 50% degraded after 1.5 min and completely degraded after 8 min. Calculated in this manner, the loss of eIF-3 activity due to treatment with trypsin correlated well with the loss of the 116 000-dalton polypeptides and one of the 36 000-dalton polypeptides. This agrees well with the low activity of the CM-50 fraction of eIF-3 in which the amounts of pp116 and pp36 are diminished to a greater extent than the other polypeptides. It is of interest to note that degradation of eIF-3 with trypsin did not result in an increase in the amounts of any of the lower molecular weight polypeptides present in the native eIF-3. These data indicate that the lower molecular weight polypeptides are not degradation products of the high molecular weight polypeptides.

In separate experiments, samples of eIF-3 (CM-50 fraction, CM-150 fraction, and CM-150 fraction treated with trypsin for 1 min) were analyzed by glycerol gradient centrifugation as previously described (Checkley et al., 1981). eIF-3 in the CM-150 fraction migrated as a 15S particle in agreement with previous findings. eIF-3 in the CM-50 fraction and in the trypsin-treated CM-150 fraction migrated somewhat more slowly but still as large particles (13–14 S). These data indicate that the loss of eIF-3 activity is not due to dissociation of the particle.

The effect of trypsin treatment on the ability of eIF-3 to bind to 40 S ribosomal subunits was determned, and the results are shown in Table III. The amounts of eIF-3 bound to 40S

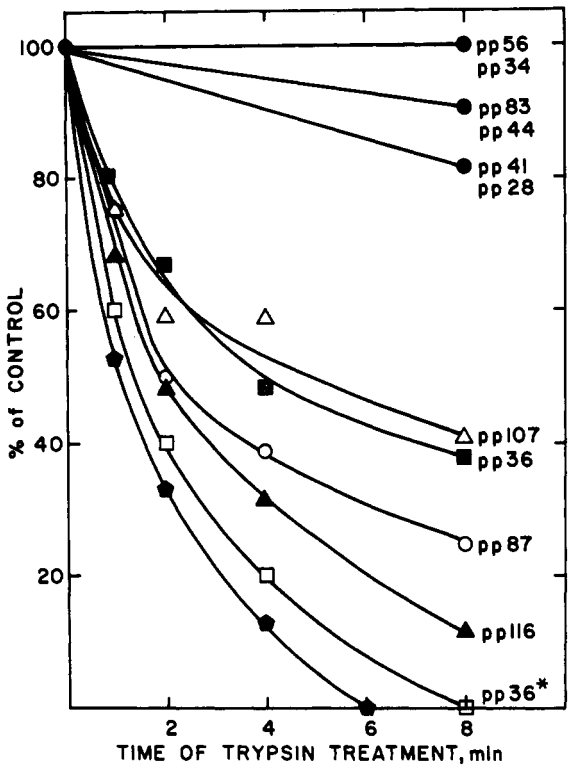

FIGURE 3: Effect of trypsin on the subunit composition of eIF-3. eIF-3 (CM-150 fraction) was treated with trypsin as described under Materials and Methods for the times indicated. Triplicate samples containing 10 μg of untreated eIF-3 or 10 μg of eIF-3 treated with trypsin for the times indicated were electrophoresed in a 12.5% SDS–polyacrylamide slab gel. The stained gel was scanned with a densitomer, and the areas under each peak were determined. Triplicate values for each polypeptide were averaged and reported as a percent of the polypeptide present in the untreated sample. (Solid pentagon) Activity of trypsin-treated eIF-3 in the in vitro polypeptide synthesis assay.

ribosomal subunits were decreased only 10–20% after treatment of the factor with trypsin for 1 min. The effect of trypsin treatment on the ability of eIF-3 to support the binding of STNV RNA (an uncapped mRNA) to 40S ribosomal subunits is shown in Table IV. Treatment of eIF-3 with trypsin for 1 min reduced its ability to support STNV RNA binding to 40S ribosomal subunits by 50%. This value correlates well with the 50% reduction in the ability of eIF-3 to support polypeptide synthesis observed after treatment with trypsin for 1 min (see Figure 2).

Discussion

Two forms of eIF-3 which differ in ability to support polypeptide synthesis were obtained from extracts of wheat germ by chromatography on CM-Sephadex. The more active form of eIF-3 is retained on CM-Sephadex in 50 mM KCl whereas the less active form is not. The less active form contains significantly reduced amounts of the 116000-dalton polypeptide (pp116) and the 36000-dalton polypeptide (pp36), presumably as a result of proteolysis by endogenous proteases. eIF-3 isolated from wheat germ by Ceglarz et al. (1980) by a procedure which does not include chromatography on CM-Sephadex also contains less than equimolar amounts of the highest molecular weight polypeptide and the polypeptide with a molecular weight of 38500. Multiple forms of eIF-3 isolated from rabbit reticulocytes have been shown to differ significantly in polypeptide composition, particularly with respect to the high molecular weight polypeptides (>100000) and a group of peptides with molecular weights between 35000 and 50000 (Meyer et al., 1980). These findings strongly suggest that some of the subunits of eIF-3 are more susceptible to proteolysis than others.

Treatment of the more active form of wheat germ eIF-3 (CM-150 fraction) with small amounts of trypsin results in a rapid degradation of four of the subunits (pp116, pp107, pp87, and pp36) and in a rapid loss in activity. The remaining six polypeptides are relatively resistant to treatment with trypsin. Trypsin treatment, at least for short periods of time, does not cause the eIF-3 particle to dissociate, and the trypsin-treated eIF-3 is still capable of binding to 40S ribosomal subunits. Trypsin treatment does, however, greatly diminish the ability of eIF-3 to support the binding of mRNA to 40S ribosomal subunits.

In summary, the data obtained in this investigation show that four of the subunits of eIF-3 (pp116, pp107, pp87, and pp36) are susceptible to proteolytic degradation, strongly suggesting that these polypeptides are in exposed positions in the eIF-3 particle. The data also indicate that pp116 and/or pp36 are essential for activity and are involved in the binding of mRNA to 40S ribosomal subunits.

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Characterization of Initiation Factor 3 from Wheat Germ. 2. Effects of Polyclonal and Monoclonal Antibodies on Activity†

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ABSTRACT: Rabbit polyclonal antibodies to wheat germ initiation factor 3 (eIF-3) were obtained and were shown to react strongly with 4 of the 10 subunits of eIF-3 (pp116, pp87, pp56, and pp36). Two mouse monoclonal antibodies were obtained, one of which reacts specifically with pp87 and one of which reacts specifically with pp36. Highly purified anti-pp87 has no effect on the activity of eIF-3. Highly purified polyclonal antibodies and anti-pp36 inhibit the ability of eIF-3 to support polypeptide synthesis in vitro and the ability of eIF-3 to support mRNA binding to 40S ribosomal subunits. These results provide additional evidence that pp116, pp87, and pp36 are in exposed positions in the eIF-3 particle and that pp36 is essential for activity.

In the preceding paper (Lauer et al., 1985), it was shown that four of the subunits of wheat germ initiation factor 3 (eIF-3)[1] (pp116, pp107, pp87, and pp36) are susceptible to proteolytic degradation, strongly suggesting that these polypeptides are in exposed positions in the eIF-3 particle. The data also indicated that pp116 and/or pp36 are essential for activity. Here we report the effects of polyclonal and monoclonal antibodies on the activity of eIF-3. The results obtained provide additional evidence that pp116, pp87, and pp36 are in exposed positions in the eIF-3 particle and that pp36 is essential for activity.

Materials and Methods

Materials. eIF-3 was prepared as described in the preceding paper (Lauer et al., 1985). Goat anti-rabbit IgG (H + L) conjugated to horseradish peroxidase and rabbit anti-mouse IgM + IgA + IgG conjugated to horseradish peroxidase, the MonoAb Id EIA kit, and ABTS were obtained from Zymed. Nitrocellulose paper was from Schleicher & Schuell, and the HRP color development reagent was from Bio-Rad Laboratories.

Assays. The abilities of eIF-3 to support polypeptide synthesis (assay A), to bind to 40S ribosomal subunits (assay B), and to support the binding of ^{125}I-labeled STNV RNA to 40S ribosomal subunits (assay C) were determined as described in the preceding paper (Lauer et al., 1985).

Production of Polyclonal Antibodies to eIF-3. A 5-lb, male New Zealand rabbit was injected intramuscularly with 0.2 mg of eIF-3 (CM-150 fraction) in 0.7 mL of Freund's complete adjuvant. Two booster injections, each containing 0.5 mg of eIF-3 in 0.75 mL of Freund's incomplete adjuvant, were given 4 and 8 weeks after the initial injection. Twelve days after the second boost, the rabbit was sacrificed, and the serum fraction of the blood was collected.

Production of Monoclonal Antibodies to eIF-3. Monoclonal antibodies were produced in the Central Hybridoma Facility at The University of Texas at Austin (director: Dr. Paul Gottlieb). A female Balb/cAM mouse was injected intraperitoneally with 0.13 mg of eIF-3 (CM-150 fraction) in 0.2 mL of Freund's complete adjuvant. A second injection in Freund's incomplete adjuvant was given 2 weeks later, and 2 weeks after the second injection, 0.2 mg of eIF-3 in buffer was injected intravenously. Three days later, the mouse was sacrificed, and the spleen was removed. Hybridomas were produced by fusion of the spleen cells with P3×63A$_G$8 myeloma cells by the procedure of Kennett et al. (1978). Twenty hybridomas producing antibodies that reacted strongly with eIF-3 in the ELISA (described below) were obtained. Ascites fluid was produced in pristane-primed Balb/c mice and used as a source of monoclonal antibodies. Monoclonal antibodies in ascites fluid produced in response to hybridomas, A8.1 and 5C-12, as well as to the parent cell, P-3, were purified as described below.

Enzyme-Linked Immunoadsorbent Assay (ELISA). The ELISA was performed by a modification of the procedure of Bahr et al. (1980). The microtiter plate wells were coated with 0.25 μg of eIF-3 in 50 μL of buffer B-100. The conjugated second antibody was either goat anti-rabbit IgG conjugated

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[1] Abbreviations: eIF-3, initiation factor 3; ABTS, 2,2′-azinobis(3-ethyl-6-benzothiazolinesulfonic acid); GMP-PNP, 5′-guanylyl imidodiphosphate; STNV, satellite tobacco necrosis virus; SDS, sodium dodecyl sulfate; ELISA, enzyme-linked immunoadsorbent assay; EITB, enzyme-linked immunoelectrotransfer blot; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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