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MUTUALLY EXCLUSIVE BINDING OF MESSENGER RNA AND INITIATOR METHIONYL TRANSFER RNA TO EUKARYOTIC INITIATION FACTOR 2

Haim Rosen and Raymond Kaempfer

Laboratory for Molecular Virology
The Hebrew University-Hadassah Medical School
Jerusalem 91000, Israel

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ABSTRACT Eukaryotic initiation factor 2 (eIF-2), purified to at least 98% homogeneity as judged by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, and containing no detectable amounts of eukaryotic initiation factor 4B (eIF-4B), is active both in the binding of Met-tRNA$_f$ and in the binding of globin mRNA. The mRNA-binding activity is completely sensitive to competitive inhibition by Met-tRNA$_f$, provided GTP is present, but not by uncharged tRNA. By contrast, binding of mRNA to partially purified eIF-4B is not inhibited by Met-tRNA$_f$. These results establish that the only mRNA-binding component in the eIF-2 preparation is eIF-2 itself, and show that a given molecule of eIF-2 can either bind to a molecule of mRNA, or form a ternary complex with Met-tRNA$_f$ and GTP, but cannot do both at once.

Eukaryotic initiation factor 2 (eIF-2) is able to reverse the block in initiation of protein synthesis observed in the absence of heme or in the presence of double-stranded RNA (1,2). This protein forms a ternary complex with Met-tRNA$_f$ and GTP and promotes binding of Met-tRNA$_f$ to the 40S ribosomal subunit (3-5), yielding a complex obligatory for binding of mRNA (5,6). In addition to these properties, eIF-2 binds to mRNA (1, 7-10), recognizing a high-affinity binding site that is also present in mRNA species lacking the 5'-terminal cap or 3'-terminal poly (A) moieties, but absent in negative-strand RNA (9). Structural analogs of the cap inhibit both the binding of Met-tRNA$_f$ and the binding of mRNA to eIF-2, and addition of eIF-2 leads to reversal of the cap analog-induced inhibition of mRNA translation (10). It was shown that mRNA, whether or not it is capped, competitively inhibits the binding of Met-tRNA$_f$ to eIF-2 (10,11). These results provided clear evidence that eIF-2 recognizes mRNA. While all eIF-2 molecules active in binding of Met-tRNA$_f$ are also capable of binding mRNA (10), the possibility still existed that some eIF-2 molecules might bind mRNA, but not Met-tRNA$_f$, or that

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the eIF-2 preparations used contained, in addition to eIF-2, a second mRNA-binding component. Here, we report that the mRNA-binding activity of eIF-2 prepared by our procedure is completely sensitive to competitive inhibition by Met-tRNA$_f$, provided GTP is present, thus establishing that the only mRNA-binding component is eIF-2 itself. These results show, furthermore, that a given molecule of eIF-2 can either engage in ternary complex formation with Met-tRNA$_f$ and GTP, or bind to mRNA, but cannot do both at once.

RESULTS

The co-purification of two characteristic activities of eIF-2, formation of a ternary complex with Met-tRNA$_f$ and GTP, and binding of mRNA, are illustrated in Fig. 1. To obtain eIF-2, the fraction of rabbit reticulocyte ribosomal wash precipitated with ammonium sulfate at 50% saturation was sub-

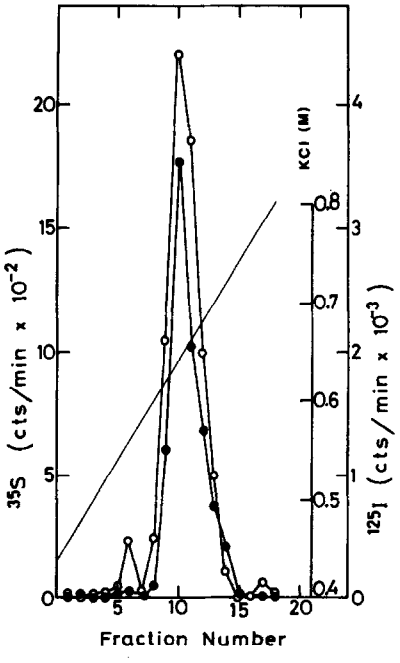

FIG. 1. *Phosphocellulose column elution profile of eIF-2.* A linear gradient of 0.4-0.8 M KCl in 50 mM Tris-HCl (pH 7.8), 0.1 mM EDTA, 1 mM dithiothreitol and 10% glycerol was used to elute eIF-2 (see text). Aliquots of 5 µl were used to assay GTP-dependent binding of ^{35}S-labeled Met-tRNA$_f$ (400 Ci/mmol; input, 2,850 cts/min) (9); a background of 37 cts/min was subtracted. Aliquots of 0.5 µl were used to assay binding of ^{125}I-labeled globin mRNA (8.6x10^5 cts/min/µg; input, 4,620 cts/min) (9); a background of 130 cts/min was subtracted. -o-o-, ^{35}S; -•-•-, ^{125}I; ———, KCl.

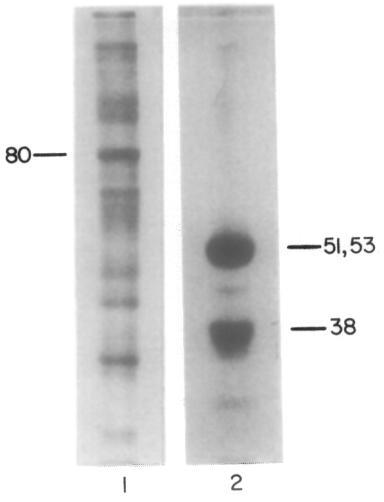

PLATE I. *Polyacrylamide gel electrophoresis of purified eIF-2.* Electrophoresis in a 10% acrylamide slab gel in the presence of 0.1% sodium dodecyl sulfate (17) was for 8 h at 30 mA. The gel was stained with Coomassie Blue. (a) *Lane 1:* material eluted between 0.2 and 0.4 M KCl from the phosphocellulose column used in Fig. 1; *lane 2:* material from pooled peak fractions 10 and 11 of the gradient shown in Fig. 1. Numbers denote molecular weight (in thousands of Mr). Reference proteins were bovine serum albumin (68,000), immunoglobulin C heavy chain (55,000) and light chain (25,000). About 150 µg of protein were placed on the gel.

jected to DEAE-cellulose chromatography, and the fraction eluted between 0.1 and 0.22 M KCl was applied to a phosphocellulose column. This column was first washed stepwise with buffer containing 0.2 M and 0.4 M KCl, and eIF-2 was then eluted by application of a linear gradient from 0.4 to 0.8 M KCl. Fig. 1 depicts the gradient portion of the column. The GTP-dependent ability of eIF-2 to bind ^{35}S-labeled Met-tRNA$_f$ is seen to co-elute with mRNA-binding activity, assayed by the retention of ^{125}I-labeled globin mRNA on nitrocellulose filters (9). Both activities elute in a single, narrow peak near 0.6 M KCl. It is worth noting that the only mRNA-binding component eluted between 0.4 and 0.8 M KCl is the one coincident with eIF-2. The protein composition of the peak fractions was analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, as shown in Plate I (lane 2). The gel was overloaded heavily to accentuate any impurities in the preparation. The band at 38,000 Mr and the heavier one at 51-53,000 Mr represent the three subunits of eIF-2; the two large subunits are not well-resolved in this

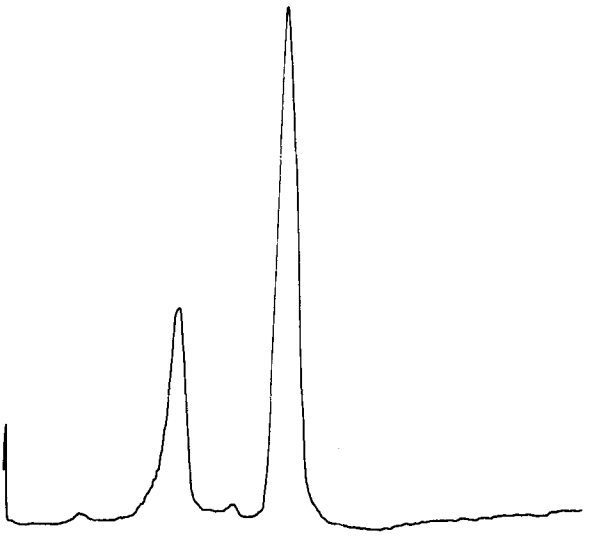

FIG. 2. *Scan of the gel of purified eIF-2 shown in Plate I, lane 2.* The top of the gel is on the right.

system (12,8,13). Although some contamination is observed, none is detectable at 80,000 Mr, the position of initiation factor eIF-4B (13). This factor is eluted from phosphocellulose near 0.3 M KCl (13), and may be represented by the strong band at 80,000 Mr in Plate I (lane 1), which depicts the gel pattern of the proteins eluted between 0.2 and 0.4 M KCl. A scan of the gel in lane 2 is shown in Fig. 2. It shows that the eIF-2 preparation is at least 98% pure.

The mRNA-binding property of this eIF-2 preparation is examined in Fig. 3a. Binding of ^{125}I-labeled globin mRNA to a limiting amount of eIF-2 was studied by retention on nitrocellulose filters. It was shown elsewhere that this binding is first-order in eIF-2 (9) and that ^{125}I-labeled and non-labeled globin mRNA bind with equal affinity to eIF-2 (14). It is seen in Fig. 3a that binding of globin mRNA to eIF-2 can be inhibited competitively, and completely, by increasing amounts of Met-tRNA$_f$, but not by equivalent amounts of uncharged tRNA. The competition by Met-tRNA$_f$ is dependent upon the presence of GTP. GTP alone does not inhibit (10). This result shows, first, that the only mRNA-binding component present in the eIF-2 preparation

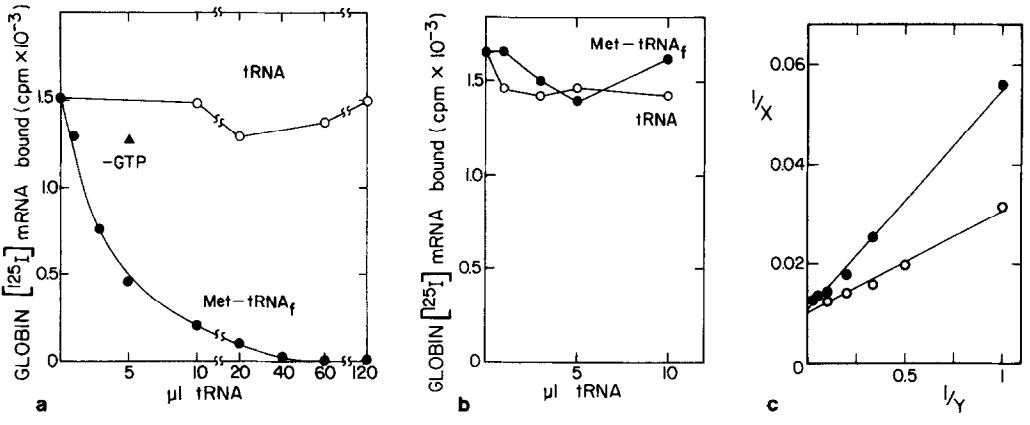

FIG. 3. *Globin mRNA binding to eIF-2: competitive inhibition by Met-tRNA$_f$.* Reaction mixtures (150 µl) contained 150 mM KCl, 20 mM Tris-HCl (pH 7.4), 0.1 mM GTP, 0.1 mM $MgCl_2$, 10 mM 2-mercaptoethanol, ^{125}I-labeled globin mRNA (4.8×10^5 cts/min/µg; input, 2,700 cts/min), the indicated amounts of a 0.1 mg/ml solution of mouse liver tRNA (charged in the presence or absence of unlabeled L-methionine with *Escherichia coli* synthetase (16); charging was monitored in a parallel sample containing ^{35}S-labeled methionine) and purified eIF-2 (fraction 10 of Fig. 1) (6 ng) (a), or material eluted from phosphocellulose between 0.2 and 0.4 M KCl (1.2 µg) (b). After incubation for 7.5 min at 30°C, samples were passed through nitrocellulose filters as in the Met-tRNA$_f$-binding assay (9). Background without protein (120 cts/min) was subtracted. In a control, 2,770 cts/min of [^{35}S]Met-tRNA$_f$ were included in (b) instead of unlabeled Met-RNA$_f$, and 2,840 cts/min were recovered after incubation by precipitation with cold trichloroacetic acid. Panel (c) depicts Lineweaver-Burk plots for the inhibition curve of Fig. 3a (•) (x denotes ^{125}I-labeled globin mRNA, y denotes unlabeled Met-tRNA$_f$) and for the reciprocal experiment (o) (x denotes ^{35}S-labeled Met-tRNA$_f$, y denotes unlabeled globin mRNA; calculated from data of ref. 10).

is eIF-2 itself. Second, it shows that a molecule of eIF-2 capable of binding to globin mRNA also can bind Met-tRNA$_f$ and GTP, but cannot do so simultaneously.

Whilst the crude eIF-4B preparation shown in Plate I (lane 1) also is able to retain globin mRNA on nitrocellulose filters, this binding is not sensitive to competition by Met-tRNA$_f$ (Fig. 3b). Controls show that Met-tRNA$_f$ is not hydrolyzed during this experiment.

DISCUSSION

These results complete the proof that the mRNA-binding activity of eIF-2, as prepared in our procedure, is a property of eIF-2 itself and is not caused by contaminating eIF-4B, or other initiation factors. Elsewhere, we show that eIF-2 prepared in this manner is able to relieve translational competition

between different mRNA species, and that a direct correlation exists between the affinity of a given mRNA for eIF-2, and its ability to compete in translation (15; H. Rosen, G. Di Segni and R. Kaempfer, in preparation).

As shown here, Met-tRNA$_f$ competitively inhibits the binding of mRNA to eIF-2. This result, and the reciprocal observation that mRNA can competitively inhibit ternary complex formation between Met-tRNA$_f$, GTP and eIF-2 (10), demonstrate that mRNA and Met-tRNA$_f$ are mutually exclusive in their binding to eIF-2. Whether in the cell a molecule of eIF-2 will form a complex with Met-tRNA$_f$ and GTP, or bind to mRNA, will depend on the equilibrium conditions. If the molar concentrations of Met-tRNA$_f$ and GTP in the cytosol exceed that of mRNA, as seems likely, free eIF-2 molecules will tend to form ternary complexes rather than bind to mRNA directly. Once the ternary complex, eIF-2•Met-tRNA$_f$•GTP is bound to the 40S ribosomal subunit, however, the interaction of a molecule of mRNA with eIF-2 may well displace the bound Met-tRNA$_f$ from this factor. Thus, during initiation of protein synthesis three processes may occur in one step: binding of mRNA to eIF-2, displacement of Met-tRNA$_f$ from eIF-2, and base-pairing between mRNA and Met-tRNA$_f$.

ACKNOWLEDGMENTS

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