Eukaryotic initiation factor 2 (eIF-2) is shown to bind ATP with high affinity. Binding of ATP to eIF-2 induces loss of the ability to form a ternary complex with Met-tRNAf and GTP, while still allowing, and even stimulating, the binding of mRNA. Ternary complex formation between eIF-2, GTP, and Met-tRNAf is inhibited effectively by ATP, but not by CTP or UTP. Hydrolysis of ATP is not required for inhibition, for adenyl-5'-yl imidodiphosphate (AMP-PNP), a nonhydrolyzable analogue of ATP, is as active an inhibitor; adenosine 5'-O-(thiotriphosphate) (ATPγS) inhibits far more weakly. Ternary complex formation is inhibited effectively by ATP, dATP, or ADP, but not by AMP and adenosine. Hence, the γ-phosphate of ATP and its 3'-OH group are not required for inhibition, but the β-phosphate is indispensable. Specific complex formation between ATP and eIF-2 is shown 1) by effective retention of Met-tRNAf and mRNA-binding activities on ATP-agarose and by the ability of free ATP, but not GTP, CTP, or UTP, to effect elution of eIF-2 from this substrate; 2) by eIF-2-dependent retention of [γ-32P]ATP or dATP on nitrocellulose filters and its inhibition by excess ATP, but not by GTP, CTP, or UTP. Upon elution from ATP-agarose by high salt concentrations, eIF-2 recovers its ability to form a ternary complex with Met-tRNAf and GTP. ATP-induced inhibition of ternary complex formation is relieved by excess Met-tRNAf, but not by excess GTP or guanylyl-5'-yl imidodiphosphate (GMP-PNP). Thus, ATP does not act by inhibiting binding of GTP to eIF-2. Instead, ATP causes Met-tRNAf in ternary complex to dissociate from eIF-2. Conversely, affinity of eIF-2 for ATP is high in the absence of GTP and Met-tRNAf (Kd ≤ 10⁻¹² M), but decreases greatly in conditions of ternary complex formation. These results support the concept that eIF-2 assumes distinct conformations for ternary complex formation and for binding of mRNA, and that these are affected differently by ATP. Interaction of ATP with an eIF-2 molecule in ternary complex with Met-tRNAf and GTP promotes displacement of Met-tRNAf from eIF-2, inducing a state favorable for binding of mRNA. ATP may thus regulate the dual binding activities of eIF-2 during initiation of translation.

A distinctive feature of eukaryotic translation is the requirement for ATP during polypeptide chain initiation (1-3). In the absence of ATP, binding of mRNA to a 40 S ribosomal subunit does not occur, even in the presence of a nonhydrolyzable analogue of GTP (2). ATP is thus not merely required to regenerate GTP (4, 5), but must have a more direct function in the binding of mRNA. A role for ATP in the recognition and binding of mRNA by eukaryotic initiation factor proteins was first suggested by the observation that binding of mRNA to eIF-4A, eIF-4B, and eIF-4F, as judged by retention of labeled mRNA on nitrocellulose filters, is ATP-dependent (6, 7). ATP stimulates the cross-linking of these initiation factors to the 5' terminal cap structure of oxidized mRNA (6-8). Both binding of mRNA and cross-linking to the cap are inhibited by the cap analogue m7GDP and do not occur if AMP-PNP is substituted for ATP (6-8), supporting the view that these factors interact with the 5' terminal cap of mRNA during initiation of translation, hydrolizing ATP in the process. Indeed, eIF-4A and eIF-4F contain an ATPase activity that can be stimulated by RNA, although a specific stimulation by mRNA is not apparent (9). Formation of a 40 S initiation complex containing mRNA and the above-mentioned initiation factors could not be demonstrated (9); the only initiation factors whose component polypeptides are present in stoichiometric amounts in 40 S met-tRNAf-mRNA complexes are eIF-4C, eIF-3, and eIF-2 (3, 10).

Eukaryotic initiation factor 2 (eIF-2) possesses dual binding activities: it forms a ternary complex with Met-tRNAf and GTP on one hand (11, 12), and it binds to a specific site in mRNA molecules on the other (13-16). The ternary complex between eIF-2, Met-tRNAf, and GTP joins to the 40 S ribosomal subunit, yielding an obligatory intermediate for the binding of mRNA at initiation of translation (17, 18). This unique property of recognizing Met-tRNAf, and placing it on the 40 S ribosomal subunit, already imparts on eIF-2 a crucial role in the binding of mRNA, a key step in translational control of eukaryotic gene expression. However, eIF-2 can also undergo a direct and specific interaction with mRNA (13-16, 19-22). All mRNA species tested possess a high-affinity binding site for eIF-2 (13), while RNA species not serving as mRNA, such as negative-strand viral RNA (13), tRNA, or ribosomal RNA (20), do not contain such a site. In satellite tobacco necrosis virus RNA (15) and mengovirus RNA (16), eIF-2 by itself recognizes and protects a nucleotide sequence that is virtually identical with the ribosome-binding site sequences in these mRNA templates. These findings suggest that eIF-2 may interact directly with mRNA during

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1 The abbreviations used are: eIF, eukaryotic initiation factor; Met-tRNAf, methionyl-tRNAf; dsRNA, double-stranded RNA; AMP-PNP, adenylyl-5'-yl imidodiphosphate; ATPγS, adenosine 5'-O-(thiotriphosphate); GMP-PNP, guanylyl-5'-yl imidodiphosphate.
its binding to the 40 S ribosomal subunit, and that this interaction may be of major importance in guiding the ribosomal subunit to its binding site in mRNA during initiation of protein synthesis. In support of this concept, studies of translational competition have shown that eIF-2 is able to relieve such competition and have revealed a direct correlation between the ability of a given mRNA species to compete at initiation of translation and its affinity for eIF-2 (23-25). Moreover, sensitivity of a given mRNA template to translational inhibition by dsRNA, which involves the inactivation of eIF-2 (19), can be correlated with the relative affinities of mRNA and dsRNA for eIF-2 (26); translation of globin mRNA, which binds more weakly than dsRNA to eIF-2, is sensitive to inhibition by dsRNA, while translation of mengovirus RNA template, which binds more tightly than dsRNA to eIF-2, is resistant. These observations suggested that interference with the interaction between mRNA and eIF-2 may lead to loss of eIF-2 activity by subsequent phosphorylation (26).

Messenger RNA and Met-tRNAf are mutually exclusive in their binding to eIF-2. That is, ternary complex formation between eIF-2, Met-tRNAf, and GTP is competitively inhibited by mRNA (21, 26-28). Conversely, binding of mRNA to eIF-2 is sensitive to inhibition by Met-tRNAf, provided GTP is present (22).

These studies of eIF-2 function emphasize the importance of the interaction between eIF-2 and mRNA for translational control, but they do not explain how the dual binding activities of eIF-2 are regulated. It was proposed that upon binding to a 40 S initiation complex containing eIF-2 and Met-tRNAf, mRNA interacts with eIF-2 and displaces Met-tRNAf from this factor (22, 29), but details of such a mechanism, if correct, are not yet understood. In particular, it is not clear how, during initiation of translation, the transition of eIF-2 from a Met-tRNAf-binding protein to an mRNA-binding protein would be accomplished.

Here, we show that eIF-2 possesses a specific, high-affinity binding site for ATP. Binding of ATP to eIF-2 leads to inhibition of ternary complex formation between eIF-2, Met-tRNAf, and GTP. By contrast, binding of mRNA to eIF-2 is not inhibited. The effect of ATP on eIF-2 does not require hydrolysis of ATP, in contrast to that on eIF-2A, eIF-4B, and eIF-4F. Our results support the concept that eIF-2 assumes distinct conformational states for ternary complex formation and for binding of mRNA, and that these states are affected differently by ATP. We show that interaction of ATP with an eIF-2 molecule in ternary complex with Met-tRNAf and GTP promotes the displacement of Met-tRNAf, inducing in eIF-2 a conformation that is favorable for the binding of mRNA.

**EXPERIMENTAL PROCEDURES**

**Materials**—ATP-agarose (1-3 μmol of 5’-ATP/ml of packed gel; Sigma); [α-32P]ATP and [α-35S]ATP (Amersham Corp.); ATP, dATP, ADP, AMP, AMP-PNP, ATPyS, UTP, CTP, GTP, and GMP-PNP (Roehminger Mannheim) were in sodium salt form and for binding of mRNA, and that these states are affected differently by ATP. We show that interaction of ATP with an eIF-2 molecule in ternary complex with Met-tRNAf and GTP promotes the displacement of Met-tRNAf, inducing in eIF-2 a conformation that is favorable for the binding of mRNA.

**Preparation of 5’-End-labeled mRNA Probe**—dsRNA was isolated from CaCl2-purified virus present in Penicillium chrysogenum, dephosphorylated with calf intestinal phosphatase, and phosphorylated using [γ-32P]ATP and T4 polynucleotide kinase in the presence of 10% dimethyl sulfoxide as described (31). The 32P labeled dsRNA migrated in polyacylamide gels as three closely spaced bands, in the same positions as the unlabeled starting material. The labeled RNA (specific activity, 0.1-2 x 106 cpm/μmol ends) was denatured by incubation for 3 min in boiling water and rapid cooling to 0 °C immediately before addition to the binding reaction mixture. At saturating levels of eIF-2, about 70% of total input radioactivity could be retained on nitrocellulose membrane filters. The single-stranded 5’-end-labeled RNA molecules generated upon denaturation bind to eIF-2 with significantly higher affinity than does native dsRNA (31). Moreover, while eIF-2 effectively protects 5’-end label in dsRNA against pancreatic ribonuclease digestion, it fails to protect 5’-end label in denatured dsRNA (31).

**Assay for Binding of mRNA to eIF-2**—Binding was assayed by retention of labeled RNA on nitrocellulose membrane filters, as described previously (24, 30). Subsaturating amounts of eIF-2 were chosen such that about 40-50% of input radioactivity was retained in the presence of eIF-2. Background binding values without protein (1-5% of the input radioactivity, depending on the age of the labeled RNA preparation) were subtracted.

**Assay for Ternary Complex Formation by eIF-2-[35S]Met-tRNAf preparation) were assayed and ternary complex formation with eIF-2 and GTP was assayed as described (13). Assay mixtures contained 0.1 mM GTP and 0.1 mM MgCl2. Amounts of eIF-2 were chosen such that about 50% of input radioactivity was retained on nitrocellulose membrane filters in the presence of eIF-2. Background binding values without protein (3-6% of the input radioactivity, depending on the [35S]Met-tRNAf preparation) were subtracted.

**Filter Retention Assay for Binding of ATP**—The procedure for assay of eIF-2-dependent retention of labeled mRNA on nitrocellulose filters was followed (24, 30), except that [α-35S]ATP or [α-32P]dATP was substituted for mRNA.

**RESULTS**

Heat denaturation of 5’-end-labeled dsRNA from P. chrysogenum yields labeled RNA molecules that bind to eIF-2 in a manner distinct from that seen for native dsRNA: binding of such single-stranded RNA molecules is with far greater affinity and occurs only at internal regions of the RNA, whereas binding of eIF-2 to dsRNA can occur at many sites along the helix, including the 5’-ends (31). Fig. 1 illustrates two characteristics of the interaction between eIF-2 and this 5’-end-labeled RNA that closely resemble the binding of
mRNA (21, 22, 26-28): binding is competed by unlabeled globin mRNA (A), as well as by Met-tRNAi in the presence of GTP (B). The affinities of the 32P-labeled probe and of globin mRNA for eIF-2 differ no more than 2-fold (A). These properties suggest the use of such 5'-end-labeled RNA molecules as a convenient and representative probe for binding of mRNA to eIF-2.

**ATP-induced Inhibition of Ternary Complex Formation between eIF-2, Met-tRNAi, and GTP**—Fig. 3A depicts the effect of increasing concentrations of ATP on ternary complex formation between eIF-2, Met-tRNAi, and GTP. Binding of Met-tRNAi is inhibited severely, the extent of inhibition exceeding 90%. By contrast, binding of mRNA, as studied with the 5'-end-labeled probe, is resistant to inhibition and is even slightly stimulated by ATP.

**ATP-induced Stimulation of mRNA Binding to eIF-2**—In Fig. 2, a weak stimulation of complex formation between eIF-2 and mRNA is seen in the presence of ATP. This stimulation becomes more pronounced in preparations of eIF-2 that exhibit lower mRNA-binding activity after storage at 4 °C. ATP strongly stimulates binding of mRNA to such eIF-2 preparations (Fig. 3, A and B), the extent of stimulation by ATP reaching 10-fold in Fig. 3A.

**Structural Features of ATP Essential for Its Inhibitory Effect on eIF-2**—As seen in Fig. 4A, GTP-dependent binding of Met-tRNAi to eIF-2 is inhibited by ATP, but not by UTP or CTP. Failure of UTP and CTP to cause inhibition suggests that chelation of Mg2+, required for ternary complex formation (12, 17), by nucleoside triphosphates cannot explain the observed inhibition by ATP. Indeed, the inhibition by 1 mM ATP is not reversed by Mg2+ concentrations ranging from 0.1 to 4 mM (data not shown). Even though Mg2+ progressively inhibits ternary complex formation (32), some relief should have been observed within this range if inhibition by ATP were due to chelation of Mg2+.

The ATP preparation did not contain detectable GDP levels as judged by thin layer chromatography; thus, contaminating traces of GDP cannot account for the observed inhibition.

As seen in Fig. 4A, the nonhydrolyzable β-imido analogue of ATP, AMP-PNP, is as effective as ATP in exerting inhibition. Hence, hydrolysis of ATP is not required for the inhibitory effect, eliminating the possibility that the observed inhibition could be due to phosphorylation of eIF-2 by contaminating traces of eIF-2 kinase.

As seen in Fig. 4B, ATP, ADP, and AMP are equally active as inhibitors. By contrast, AMP is only weakly inhibitory and adenosine fails to inhibit at all. Thus, the γ-phosphate of ATP is not required for effective inhibition of ternary complex formation, but the β-phosphate is indispensable.

While the β-imido analogue of ATP, AMP-PNP, inhibits ternary complex formation as actively as ATP (Fig. 4A), another nonhydrolyzable analogue, ATPγS, displays little, if any, inhibitory activity (Fig. 4B).

**Failure of GTP to Relieve ATP-induced Inhibition of Ternary Complex Formation**—The finding that ATP inhibits the GTP-dependent binding of Met-tRNAi to eIF-2 but does not inhibit the binding of mRNA (Fig. 2) might suggest that ATP could act by competing for the binding site of GTP. The data of Fig. 5 do not support this possibility. As seen in Fig. 5, A and B, the inhibitory effect of ATP on ternary complex formation is not perceptibly relieved by GTP. This lack of relief by GTP is not due to contaminating GDP, which binds to eIF-2 more tightly than ATP and inhibits ternary complex formation (5): GMP-PNP, a nonhydrolyzable analogue of GTP that can replace GTP in ternary complex formation, also fails to relieve the inhibition by ATP (Fig. 5, A and B).

**Retention of eIF-2 on ATP-Agarose and Elution by ATP**—The foregoing results suggest that eIF-2 interacts with ATP. Direct evidence for this concept is provided by the data of Fig. 6. In the experiment of panel A, purified eIF-2 was applied to an ATP-agarose column. Initiation factor eIF-2 activity, monitored by ternary complex formation with 35S-labeled Met-tRNAi and GTP, as well as by binding of the 32P-labeled mRNA probe, is retained on this column and upon application.
Demonstration of a Specific Complex between eIF-2 and ATP—Label in [$\alpha$-$^{32}$P]dATP is displaced to the excluded fraction when first incubated with eIF-2 and then subjected to chromatography on Sephadex G-50 (data not shown). As seen in Fig. 7, the ATP-eIF-2 complex is sufficiently stable to survive nitrocellulose filtration. Progressive amounts of label in [$\alpha$-$^{32}$P]dATP (Fig. 7A) or [$\alpha$-$^{32}$P]ATP (Fig. 7B) are retained on such filters in response to increasing amounts of eIF-2 (upper curve). Binding is specific, as it is competed by excess unlabeled ATP or dATP, but not by GTP, UTP, or CTP. Use of $\alpha$-labeled ATP eliminates phosphorylation as explanation for the retention of label. These results confirm the finding of Fig. 4A and show that eIF-2 interacts tightly with both ATP and dATP.

Scatchard analysis of the ATP-binding curve of Fig. 8 shows a binding constant of $10^{-12}$ M. In the absence of other components for protein synthesis, ATP thus binds with very high affinity to eIF-2.

Dissociation of Ternary Complex by ATP—In Fig. 9, kinetics of ternary complex formation were studied. While ATP severely inhibited ternary complex formation when added at zero time, its addition at 15 min led to an immediate and extensive decline in [$\beta$-$^{35}$S]Met-tRNA already bound in ternary complex. This result shows that ternary complexes, formed of a salt gradient is eluted at high concentration (1 M KCl). Met-tRNA, and mRNA binding activities are co-eluted. The recovery of Met-tRNA binding activity shows that eIF-2 regains its ability to form a ternary complex once it is removed from ATP.

These observations suggest that it should be possible to elute eIF-2 from ATP-agarose with buffer containing ATP. As seen in Fig. 6B, ATP was unable to effect elution of eIF-2 at 500 mM KCl. By contrast, at 500 mM KCl, bound factor activity was eluted as soon as ATP was applied. This elution behavior points to a high affinity of eIF-2 for ATP. Apparently, bound ATP is not readily exchanged. Only mRNA binding activity was assayed in this case, since the presence of ATP precluded measurement of Met-tRNA binding activity (Fig. 2). In the experiment of Fig. 6C, CTP, UTP, or GTP failed to cause elution of eIF-2 activity, while ATP at the same concentration was effective. This result demonstrates specificity in the binding of ATP to eIF-2.

In C, over 80% of input eIF-2 activity could be recovered from ATP-agarose upon elution with salt and/or ATP.
allowed to proceed for 15 min in the absence (0) or presence of 2 mM ATP (A). The reaction mixture that did not receive ATP was then split; one-half received 2 mM ATP (B), while the other half did not (0). At the indicated times, aliquots were removed, and 32P label in ternary complex was determined.

Inhibition of ternary complex formation by ATP is not relieved by excess Met-tRNA, while the other half did not (0). ATP and Met-tRNA, are present, binding of ATP to eIF-2 is severely inhibited. Thus, affinity of eIF-2 for ATP is very high in the absence of GTP and Met-tRNA, (Fig. 8) but decreases greatly in conditions of ternary complex formation (Fig. 11).

Relief of ATP-induced inhibition of ternary complex formation by excess Met-tRNA. Ternary complex formation between 10 ng of eIF-2, GTP, and [35S]Met-tRNA was assayed in the absence (0) or presence (0) of 3 mM ATP. The amount of [35S]Met-tRNA in the reaction mixtures was increased beyond standard assay conditions (3650 cpm; 2 x 105 cpm/pmol, 730 cpm/nl) as shown; ×, background radioactivity bound in the absence of eIF-2.

Decreased affinity of ATP for eIF-2 in the presence of Met-tRNA and GTP. Binding of [32P]ATP to eIF-2 was assayed as for Fig. 8 (0) and also in the presence of GTP (0), unlabeled Met-tRNA, or both (0). GTP and Met-tRNA were added at the concentrations used for ternary complex formation.

in the absence of ATP, rapidly dissociate in the presence of ATP. This destabilization by ATP can account for the observed inhibition of ternary complex formation. The destabilizing effect of ATP is not relieved by simultaneous addition of excess GTP or GMP-PNP (not shown).

Effect of Met-tRNA, and GTP on Binding of ATP to eIF-2—At low concentrations, binding of labeled ATP to eIF-2 is not affected by GTP and is only marginally inhibited by Met-tRNA. (Fig. 11). However, when both GTP and Met-tRNA, are present, binding of ATP to eIF-2 is severely inhibited. Thus, affinity of eIF-2 for ATP is very high in the absence of GTP and Met-tRNA, (Fig. 8) but decreases greatly in conditions of ternary complex formation (Fig. 11).

DISCUSSION

Our experiments show that eIF-2 possesses a specific, high- affinity binding site for ATP. This conclusion is based on several lines of evidence. ATP is able to inhibit ternary complex formation between eIF-2, GTP, and Met-tRNA, (Fig. 2). Neither CTP nor UTP possesses this property (Fig. 4A). The inhibition is not due to chelation of Mg2+ or contaminating GDP. Direct evidence for specific binding of eIF-2 to ATP is furnished, on one hand, by effective retention of its Met-tRNA, and mRNA binding activities on ATP-agarose (Fig. 6A), and by the ability of ATP, but not of GTP, CTP, or UTP, to effect elution of eIF-2 from this substrate (Fig. 6, B and C). On the other hand, formation of a tight complex between [32P]ATP and eIF-2 can be detected by nitrocellulose filter retention analysis (Figs. 7 and 8). Binding of labeled ATP to eIF-2 is inhibited by excess unlabeled ATP, but not by GTP, CTP, or UTP (Fig. 7B).

The inhibitory effect of ATP on eIF-2 does not require the hydrolysis of ATP, since the nonhydrolyzable analogue, AMP-PNP, inhibits ternary complex formation as effectively (Fig. 4A). ATP-Y1, on the other hand, exhibits little, if any, inhibitory activity (Fig. 4B). Apparently, eIF-2 is sensitive to the difference in bond angles between the ψ- and γ-phosphates in these two nonhydrolyzable analogues. While the γ-phosphate of ATP is not required for its inhibitory effect on ternary complex formation, the ψ-phosphate is indispensable (Fig. 4B). dATP is also able to inhibit ternary complex formation (Fig. 4A) and to bind tightly to eIF-2 (Fig. 7). Apparently, the 3'-hydroxyl group of the ribose moiety of ATP is not essential for recognition by eIF-2.

Although ATP has been shown to participate in the action of other initiation factors for eukaryotic protein synthesis, eIF-4A, eIF-4B, and eIF-4F (6–9), our results strongly support the concept that the interaction of ATP with eIF-2 is distinct from that with those factors. An activity specifically inherent to eIF-2, the formation of a ternary complex with Met-tRNA, and GTP, is inhibited by ATP and retained on ATP-agarose (Figs. 3, 4, and 6). While eIF-2 forms a stable complex with ATP (Figs. 6–8), formation of a stable complex between ATP and eIF-4A, eIF-4B, or eIF-4F has not been demonstrated. Most critically, ATP hydrolysis is not required for the inhibitory effect of ATP on ternary complex formation by eIF-2 (Fig. 4), yet it is absolutely needed for the action of eIF-4A, eIF-4B, and eIF-4F (6–8).

While ATP inhibits the binding of Met-tRNA to eIF-2, it does not inhibit the binding of mRNA (Fig. 2) and can even stimulate it extensively (Fig. 3). The finding that ATP inhibits the GTP-dependent binding of Met-tRNA to eIF-2, but does not inhibit the GTP-independent binding of mRNA, might suggest that ATP prevents the binding of GTP. As shown in Fig. 5, however, increasing concentrations of either GTP or its nonhydrolyzable analogue, GMP-PNP, fail to relieve the inhibition of ternary complex formation by ATP. Hence, ATP does not inhibit ternary complex formation by merely occupying the GTP-binding site. By contrast, inhibi-
tion of ternary complex formation by ATP is readily reversed by excess Met-tRNAf (Fig. 10), suggesting that ATP interferes with the interaction between eIF-2 and Met-tRNAf. These results strongly support the concept that ATP inhibits ternary complex formation by either occupying or altering the conformation of the binding site for Met-tRNAf in eIF-2.

Several arguments do not favor the interpretation that the eIF-2 population is composed of two classes, differing by the presence of ATP: (i) eIF-2 molecules lacking ATP, active in ternary complex formation, and (ii) those carrying ATP, active in binding of mRNA. First, mRNA and Met-tRNAf compete in binding to eIF-2 (21, 22, 26-28) and the binding of mRNA can be completely prevented by competing amounts of Met-tRNAf, provided GTP is present (Fig. 1B; Ref. 25). Second, as seen in Fig. 6A, eIF-2 molecules eluted from ATP-agarose with a salt gradient are active in binding of mRNA as well as in binding of Met-tRNAf. The fact that eIF-2 can be eluted effectively from ATP-agarose by buffer containing ATP (Fig. 6, B and C) implies that eIF-2 molecules carrying free ATP are not retained on the column. Hence, the eIF-2 molecules eluted by high salt concentrations, rather than by ATP, in Fig. 6A should be free of ATP. It can be concluded, therefore, that eIF-2 molecules lacking ATP can bind either to Met-tRNAf or to mRNA.

The recovery of Met-tRNAf binding activity from an ATP-agarose column shows that upon dissociation from ATP, eIF-2 regains its ability to form a ternary complex. This and the finding that hydrolysis of ATP is not required for inhibition of eIF-2 activity support the concept that binding of ATP to eIF-2 induces a reversible conformational change in the protein that leads to loss of the ability to form a complex with Met-tRNAf and GTP, while still allowing the binding of an mRNA molecule.

Our results suggest that ATP stimulates the binding of mRNA to eIF-2 in particular when its conformation has been altered, for example, upon storage (Fig. 3). ATP, upon binding to eIF-2, appears to drive the protein back into the active state for binding of mRNA. Another condition that affects the binding of mRNA to eIF-2 is the presence of GTP and Met-tRNAf (Fig. 1B). As stated above, mRNA and Met-tRNAf are mutually exclusive in their binding to eIF-2. ATP may profoundly affect this property since it induces in eIF-2 a state that is favorable for binding of mRNA, but unfavorable for ternary complex formation with Met-tRNAf and GTP. Our findings thus suggest a regulatory role for ATP through its interaction with eIF-2, in initiation of eukaryotic translation and specifically, in the binding of mRNA. Binding of ATP to eIF-2 in ternary complex with Met-tRNAf and GTP may effect the conversion of eIF-2 from a Met-tRNAf-binding protein to an mRNA-binding protein.

The question now arises how ternary complex formation can take place at intracellular concentrations of ATP of 2–3 mM, when ATP-eIF-2 complex formation exhibits a Ka ≤ 10^18 M in the absence of other components for protein synthesis (Fig. 8). As seen in Fig. 11, however, in the presence of GTP and Met-tRNAf at concentrations optimal for ternary complex formation, the affinity of ATP for eIF-2 is greatly decreased. This explains why relatively high concentrations of ATP are needed to inhibit ternary complex formation. Indeed, in conditions of ternary complex formation, binding of ATP to free eIF-2 (Fig. 11) and formation of ternary complexes (33) exhibit Ka values in the same range. ATP on one hand, and Met-tRNAf/GTP on the other, thus act to inhibit each other in binding to eIF-2. When binding of ATP prevails, formation of the mRNA-eIF-2 complex is favored at the expense of ternary complex.

The respective roles in translation of the dual activities of eIF-2, to form a ternary complex with Met-tRNAf and GTP on one hand, and to form a specific complex with mRNA on the other, may now be interpreted more readily by considering the role of ATP. At physiological salt concentrations, ternary complex formation between eIF-2, GTP, and Met-tRNAf is essentially irreversible, while mRNA-eIF-2 complexes are in equilibrium with the free components (26, 27). Hence, free eIF-2 molecules are favored to form stable, ternary complexes with GTP and Met-tRNAf which bind to 40 S ribosomal subunits, an obligatory step before binding of mRNA can occur (17, 18). Our results strongly suggest that interaction of ATP with an eIF-2 molecule in ternary complex with Met-tRNAf and GTP and located on the 40 S ribosomal subunit promotes at once the displacement of Met-tRNAf from eIF-2 and the binding of mRNA to eIF-2. The mRNA molecule is recognized and bound by eIF-2 at the ribosome-binding site sequence (15, 16) and concomitantly, Met-tRNAf, and the AUG initiation codon in mRNA undergo base pairing. Since, as we have shown, the ATP molecule that binds to eIF-2 does not have to undergo hydrolysis, it may then serve other purposes, for example, to support the action of proteins that bind to the 5′-terminal cap structure of the mRNA molecule in dependence on ATP hydrolysis.

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