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A Kinase Able To Phosphorylate Exogenous Protein Synthesis Initiation Factor eIF-2α Is Present in Lysates of Mengovirus-Infected L Cells

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Infection of mouse L929 cells by mengovirus resulted in the expression of a kinase activity that selectively phosphorylated the small, 38,000-molecular-weight subunit of eucaryotic initiation factor 2 and histone H2. This kinase activity was independent of host cell RNA synthesis and was located in the postribosomal supernatant (S-100 fraction) early after infection (up to 3 h). At later times after infection (5 h), kinase activity was also associated with the polysome fraction. The kinase present in the S-100 fraction bound strongly to DEAE-cellulose, its peak activity eluting at 0.5 M KCl. Kinase activity was independent of the presence of exogenous double-stranded RNA, and KCl at concentrations greater than 0.1 M inhibited eucaryotic initiation factor 2 phosphorylation. The 67,000-molecular-weight phosphoprotein activated in interferon-treated cells by double-stranded RNA was not detected by standard phosphorylation assays in lysates from mengovirus-infected cells. Labeling of this protein in vivo during 5 h of infection was also not detected. The DEAE-cellulose-purified mengovirus kinase inhibited protein synthesis in reticulocyte lysates, and the inhibition was not reversible by high concentrations of poly(I) · poly(C).

The mechanism by which mengovirus infection leads to a rapid inhibition of cellular protein synthesis is obscure. The overall characteristics of the inhibition are typical of a block at the level of initiation of protein synthesis; i.e., polysomes disaggregate, and 80S ribosomes accumulate (for a review, see reference 16). Lysates (S-15 fractions) from mengovirus-infected cells reflect this decreased rate of protein synthesis. The postribosomal supernatant (S-100 fraction) from early-infected cells as well as the crude ribosome fraction from late-infected cells appears to be responsible for the decreased activity of the lysates (16). Washing these ribosomes with solutions containing 0.5 M KCl partially restores their activity, and the resulting ribosomal salt wash is inhibitory when included in a translation system from uninfected cells. The inhibitor present in the salt wash is micrococcal nuclease resistant and heat sensitive (16).

The present work represents a continuation of our studies directed toward understanding the mechanism of protein synthesis inhibition by mengovirus. We examined the possibility that the rapid inhibition of host protein synthesis results from the phosphorylation of a factor necessary for the initiation of protein synthesis. It is well known that in rabbit reticulocytes or interferon-treated human or mouse L-cell lysates, the addition of double-stranded RNA (dsRNA) at very low concentrations leads to the activation of an inhibitor of the initiation of protein synthesis (4, 5, 7, 9). The inhibitor is a dsRNA-dependent protein kinase which selectively phosphorylates two proteins, the small, 38,000-molecular-weight subunit of eucaryotic initiation factor 2 (eIF-2α) and a 67,000-molecular-weight protein known as dsI in reticulocytes (11, 12) or P-1 in interferon-treated mouse cells (22, 24). Both dsI and P-1 are autophosphorylated and are thought to be the kinases themselves (2, 4, 12, 22, 24).

During initiation, eIF-2 complexes with GTP and Met-tRNA$_i$ and delivers the latter to a 40S ribosomal subunit. In a subsequent step GTP is hydrolyzed and eIF-2-GDP must be reconverted to eIF-2-GTP if it is to function catalytically. The reconversion is accomplished by another factor called guanine nucleotide exchange factor (for a review, see reference 21). When eIF-2α is phosphorylated, it cannot function catalytically, presumably because it sequesters guanine nucleotide exchange factor in an inactive complex (21).

In view of the fact that mengovirus-infected cells contain replicative intermediates (17) that may activate the dsRNA-dependent kinase, we examined lysates of mengovirus-infected cells for the presence of kinase activity. We demonstrated that lysates from mengovirus-infected cells were capable of phosphorylating exogenous reticulocyte eIF-2α and histone H2. Kinase activity was present in the S-100 fraction of the cell between 1 and 3 h after infection and also in the ribosome fraction at 5 h after infection. The relationship of the kinase in mengovirus-infected cells to the inhibition of protein synthesis is discussed below.

MATERIALS AND METHODS

Cell culture conditions and virus growth. Mouse L929 cells were grown in either suspension culture in Spinner flasks or plastic Falcon culture plates, both by the method of Pensiero and Lucas-Lenard (16). The growth and purification of mengovirus was accomplished by the method of Abreu and Lucas-Lenard (1). Seed stocks of wild-type mengovirus (*is*$^+$) (13, 26) were obtained from P. I. Marcus via E. Simon, Purdue University. Titers ranged from 1×10^{10} to 3×10^{10} PFU/ml. Infection was carried out at a multiplicity of infection (MOI) of 25 PFU per cell for various times. In some experiments requiring phosphorylated P-1 and eIF-2 for gel markers, the L(Y) cell line from J. S. Youngner, University of Pittsburgh, was used. In this cell line, the interferon-induced, dsRNA-dependent kinase (IFN-dsRNA kinase) was readily induced with low doses (100 U/ml) of interferon and was strongly activated by poly(I) · poly(C).

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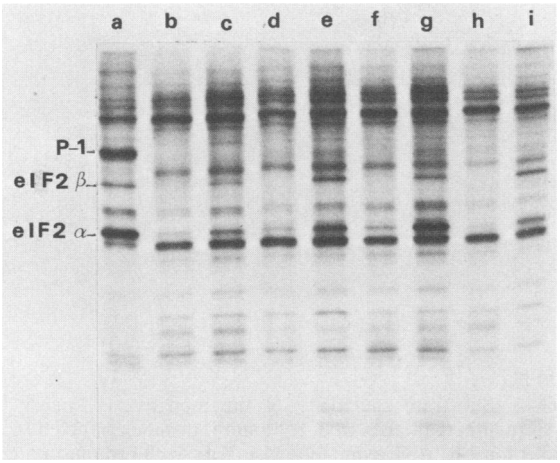

FIG. 1. eIF-2α kinase activity in the S-100 fraction from uninfected and mengovirus-infected L cells. Kinase activity was assayed as described in Materials and Methods. Lanes: a, S-15 lysate from mouse L(Y) cells pretreated with interferon plus poly(I) · poly(C) and eIF-2; b, S-100 fraction from mock-infected L cells; c, S-100 fraction from mock-infected cells plus eIF-2; d, S-100 fraction from cells infected for 1 h; e, S-100 fraction from cells infected for 1 h plus eIF-2; f, S-100 fraction from cells infected for 3 h; g, S-100 fraction from cells infected for 3 h plus eIF-2; h, S-100 fraction from cells infected for 5 h; i, S-100 fraction from cells infected for 5 h plus eIF-2.

Preparation of cell-free lysates. L929 cells were harvested and lysed by the method of Pensiero and Lucas-Lenard (16) from Spinner culture or by the lysolecithin method of Brown et al. (3) from monolayer cultures. Some monolayer cultures were preincubated with 1,000 U of mouse interferon per ml for 16 to 18 h before lysis with lysolecithin. After lysis, cell extracts (S-15) were separated into S-100 and polysome fractions. Procedures for fractionations and preparation of washed ribosomes, as well as storage conditions, were outlined by Pensiero and Lucas-Lenard (16).

Protein kinase assay. Reaction mixtures contained the following in a final volume of 15 μl: 20 mM Tris hydrochloride (pH 7.6), 80 mM KCl, 3 mM magnesium acetate, 1.5 mM dithiothreitol, 0.5 μCi of [γ-^{32}P]ATP, 25 μM ATP, and approximately 30 μg of lysate protein. Reticulocyte eIF-2 (0.3 μg) and poly(I) · poly(C) (4 ng) were included in the reaction mixture, as indicated in the figure legends.

The mixtures were incubated at 30°C for 15 min, and the reactions were terminated by the addition of an equal volume of 2× sample buffer (10). The samples were heated at 100°C for 2 min and electrophoresed on sodium dodecyl sulfate-polyacrylamide gels (10). The gels were dried and autoradiographed.

DEAE-cellulose chromatography. For purification of the mengovirus kinase, 4 ml (32 mg of protein) of S-100 fraction from 3.6×10^9 3-h infected L929 cells in suspension culture were applied to a DEAE-cellulose column previously equilibrated in starting buffer (40 mM KCl, 20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.4], 1 mM magnesium acetate, 0.5 mM dithiothreitol. For isolation of the IFN-dsRNA kinase, ribosomes from 3×10^8 interferon-treated L929 cells were washed with starting buffer containing 0.6 M KCl. The resulting salt wash (500 μl) was dialyzed for 5 h against 1 liter of starting buffer before chromatography. Fractions in each case were eluted with increasing KCl concentrations in the same buffer. The fractions were dialyzed for 4 h against a saturated ammonium sulfate solution. The precipitates were suspended in approximately 200 μl of a buffer containing 20 mM HEPES (pH 7.4), 50 mM KCl, 5 mM magnesium acetate, and 1 mM dithiothreitol and dialyzed for 6 h against the same buffer before storage at −70°C. The amount of protein recovered in the 0.5 M KCl fraction (mengovirus kinase) was 280 μg.

Protein synthesis assay in reticulocyte lysates. Micrococcal nuclease-treated rabbit reticulocyte lysate from Promega Biotec was used. The reaction mixture included (in a final volume of 25 μl) 17.5 μl of reticulocyte lysate (including energy mix, etc., as prepared by Promega Biotec), 25 μM amino acid mixture (minus methionine), 0.5 μg of brome mosaic virus RNA (Promega Biotec), 25 μCi of [^{35}S] methionine (1,200 Ci/mmol), and when used, 1.5 μg of kinase (DEAE-cellulose fraction) and 4 ng of poly(I) · poly(C). Incubation was at 30°C, and protein synthesis was assayed by analyzing 1-μl samples for radioactivity incorporated into hot 5% trichloroacetic acid-precipitable material as described previously (8).

RESULTS

Mengovirus infection of L929 cells results in the phosphorylation of eIF-2α. When incubated in the presence of [γ-^{32}P]ATP, S-15 lysates from mengovirus-infected cells demonstrated an increased ability to phosphorylate exogenous eIF-2α relative to lysates from uninfected cells (data not shown). This kinase activity was detectable as early as 1 h after infection and increased with time up to at least 5 h after infection. The S-15 lysates were subsequently separated into S-100 and polysome fractions, and each was examined for its ability to phosphorylate eIF-2α. Kinase activity was present in the S-100 fraction of cells infected for 1 to 3 h but was reduced in this fraction by 5 h after infection (Fig. 1). The S-100 fraction from cells infected for 3 h always showed the greatest amount of kinase activity. Kinase activity was absent in the polysome fraction from cells infected for 1 h; it was barely detectable in this fraction by 3 h and was strongly evident by 5 h after infection (Fig. 2).

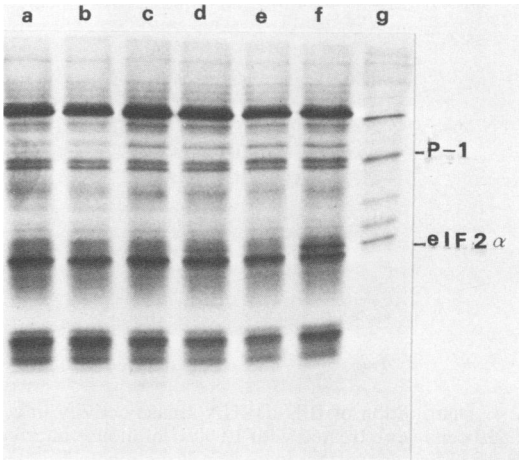

FIG. 2. eIF-2α kinase activity in the polysome fraction from infected cells. The kinase assays were performed as described in Materials and Methods. In this experiment, the polysome fraction from cells infected for the following times was examined. Lanes: a, 1 h; b, 1 h plus eIF-2; c, 3 h; d, 3 h plus eIF-2; e, 5 h; f, 5 h plus eIF-2; g, S-15 fraction from interferon-treated L(Y) cells plus poly (I) · poly(C) and eIF-2.

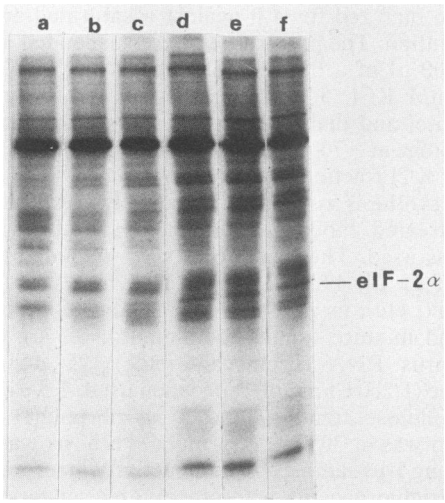

FIG. 3. The appearance of mengovirus kinase activity in the S-100 fractions of cells infected with virus at different MOIs. L929 cells were mock infected (a) or infected with mengovirus at MOIs of 1 (b), 5 (c), 10 (d), 20 (e), or 30 (f) PFU per cell. At 3 h after infection, the cells were lysed with lysolecithin (3). The crude lysates were then fractionated and the S-100 fractions (30 μg each) were tested for kinase activity as described in Materials and Methods. Each reaction mixture (15 μl) contained 0.8 μg of eIF-2. Marker eIF-2 migrated to the position indicated.

The proteins phosphorylated by S-15 lysates from interferon-treated cells in the presence of poly(I) · poly(C) are also illustrated in Fig. 1 and 2 (lanes a and g, respectively). It is evident that both eIF-2α and P-1 were phosphorylated. P-1 was not phosphorylated in lysates from mengovirus-infected cells at any of the times examined.

The effect of MOI on the appearance of the kinase in the S-100 fraction from cells infected for 3 h was also examined. No kinase activity was detected in control cells or in cells infected at an MOI of 1 PFU per cell (Fig. 3). However,

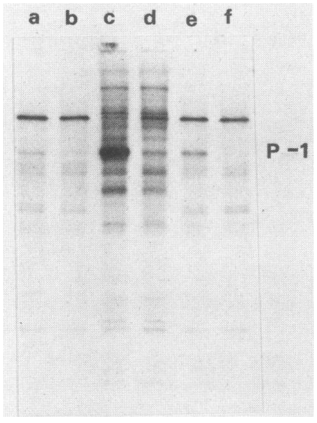

FIG. 4. Distribution of IFN-dsRNA kinase activity in cell fractions. L929 cells were treated with 1,000 U of mouse interferon per ml for 18 h. Lysates were prepared and fractionated as described previously (16). Approximately 35 μg of S-100 protein (lanes a and b), polysomal protein (c and d), or whole lysate (S-15) protein (e and f) was incubated (in a final volume of 15 μl) with [γ-^{32}P]ATP in the presence (lanes a, c, and e) or absence (b, d, and f) of 2 ng of poly(I) · poly(C) as described in Materials and Methods. Samples were analyzed by electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gels (10).

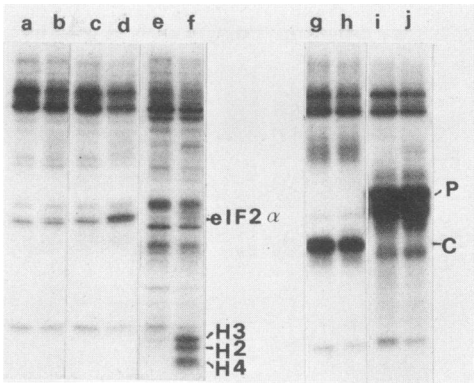

FIG. 5. Substrate specificity of the mengovirus kinase. L929 cells were infected (lanes b, d, f, h, and j) or mock infected (a, c, e, g, and i) for 3 h. Approximately 35 μg of S-100 protein in an 18-μl reaction volume was incubated with [γ-^{32}P]ATP as described in Materials and Methods in the presence of the following proteins. Lanes: a and b, no additions; c and d, 0.6 μg of reticulocyte eIF-2; e and f, 4 μg of calf thymus histones; g and h, 4 μg of casein; i and j, 4 μg of phosvitin. C, Casein (molecular weight, 23,600); P, phosvitin (molecular weight, 40,000). Samples were analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoreisis and autoradiographed. The positions of the substrates, as determined by molecular weight markers, are indicated.

eIF-2α phosphorylating activity was observed in cells infected at MOIs of 5 to 30. MOIs of greater than 10 PFU per cell did not appear to result in the activation of more kinase.

To further distinguish the mengovirus kinase activity from the IFN-dsRNA kinase, we analyzed the cellular location of the latter kinase in this particular cell line. Assays of S-15, S-100, and polysome fractions of interferon-treated cells showed that the polysome fraction had the greatest amount of kinase activity, as detected by P-1 phosphorylation (Fig. 4). This finding is in agreement with those of others (2, 9, 24).

Substrate specificity of the kinase present in the S-100 fraction of mengovirus-infected cells. Various proteins were tested for their abilities to act as substrates for the mengovirus kinase. These were phosvitin, casein, calf thymus histones, and eIF-2. Only histones and eIF-2 were selectively phosphorylated by the S-100 fraction from infected cells (Fig. 5). Initiation factors eIF-4A, eIF-4B, and eIF-4F were also tested, and these were not found to be substrates (data not shown). Further investigation using a 100-fold purified preparation of the kinase showed histone H2 to be the primary histone phosphorylated (Fig. 6B). The IFN-dsRNA kinase has been reported to phosphorylate histones (9, 24), but the most purified preparation of this kinase no longer appears to possess this activity (2).

The effect of actinomycin D on mengovirus kinase activity. The effect of actinomycin D on kinase activity in cells infected for 3 h was also examined. The phosphorylation of eIF-2α by this fraction was compared with that by a control S-100 from cells infected in the absence of actinomycin D. Actinomycin D (0.5 μg/ml) did not prevent the expression of this kinase; the ability of the fraction to phosphorylate eIF-2 remained unchanged in the presence of the drug (data not shown). It is noteworthy that RNA synthesis was reduced to the same extent (about 50% of that of mock-infected cells) by actinomycin D as by virus infection, as determined by [^{3}H]uridine incorporation into acid-insoluble material (data not shown). These results suggest that no new host RNA synthesis is necessary for expression of the kinase.

Elution characteristics of the mengovirus kinase and IFN-

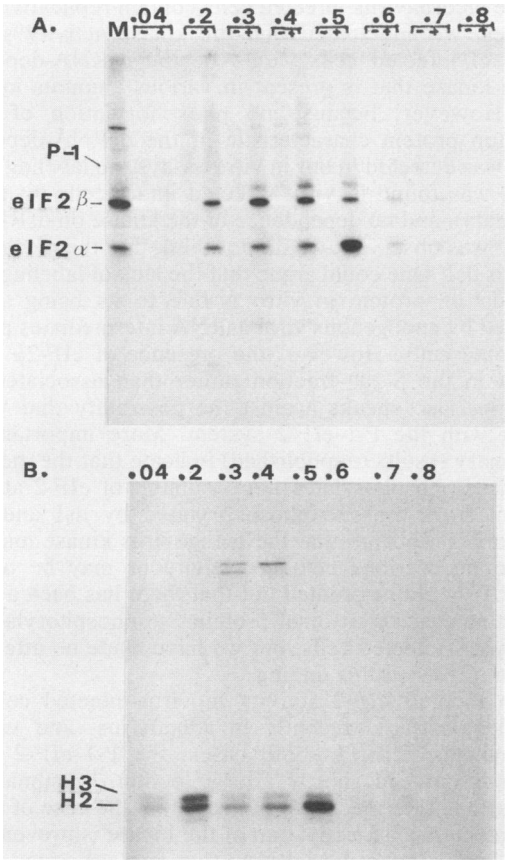

FIG. 6. Fractionation of the mengovirus kinase by DEAE-cellulose chromatography. The S-100 fraction from L cells infected for 3 h was subjected to DEAE-cellulose chromatography, and fractions were eluted stepwise with buffers containing increasing concentrations of KCl as described in Materials and Methods. (A) Approximately 1.5 μg of protein from each dialyzed fraction was incubated in a final volume of 15 μl with [γ-^{32}P]ATP with (+) or without (−) 0.6 μg of eIF-2. The salt concentration (M) used to elute each fraction is indicated above each lane. In lane M, a polysome fraction from uninfected, interferon-treated L cells was incubated in the presence of eIF-2 and poly(I) · poly(C) to indicate the positions of P-1 and eIF-2. (B) In each case, 4 μg of a histone H2-enriched fraction was used in place of eIF-2 in the assay for phosphorylation.

dsRNA kinase activities from DEAE-cellulose. The S-100 fraction from L929 cells infected for 3 h was applied to a DEAE-cellulose column (see Materials and Methods), and fractions were eluted with buffers containing increasing KCl concentrations. Fractions eluting at 0.04 to 0.8 M KCl were then tested for their abilities to phosphorylate eIF-2 and histone H2. There was kinase activity in fractions eluting at 0.2 to 0.5 M KCl, with most activity in the fraction eluting at 0.5 M KCl, as determined by eIF-2α phosphorylation (Fig. 6A). This fraction also proved to be capable of phosphorylating histone H2 (Fig. 6B). It is important to note that even with an approximately 100-fold concentration of kinase, no labeling of P-1 with [γ-^{32}P]ATP occurred.

The β subunit of eIF-2 was nearly always phosphorylated by the L929 cell lysates. The peak activity responsible for this reaction (presumably the casein kinase II [27, 28]) eluted at 0.3 M KCl (Fig. 6A). Phosphorylation of the β subunit of eIF-2 is not known to affect eIF-2 activity (27, 28).

For comparative purposes, the binding properties of the IFN-dsRNA kinase to DEAE-cellulose were also examined. The 0.6 M KCl wash of ribosomes from interferon-treated cells was subjected to DEAE-cellulose chromatography as described above. Fractions were then incubated with poly(I) · poly(C) and tested for the presence of P-1. As found by others (2, 9, 24), the IFN-dsRNA kinase activity did not adsorb to DEAE-cellulose and was recovered in the 0.04 M KCl starting buffer (data not shown). Once activated, however, the IFN-dsRNA kinase binds to DEAE-cellulose, but elutes at a lower concentration of KCl (0.3 M) than the mengovirus kinase (4).

Effect of poly(I) · poly(C) and KCl on mengovirus kinase activity. The above assays for kinase activity in lysates from mengovirus-infected cells were performed in the absence of dsRNA. Indeed, kinase activity was essentially independent of exogenous dsRNA, although there was a slight stimulation of eIF-2 phosphorylation at 0.5 μg of poly(I) · poly(C) per ml and a slight decrease in phosphorylation at 50 μg/ml (Fig. 7A). It is possible that the slight effect of dsRNA resulted from a minor contamination in this particular eIF-2 preparation by the dsRNA-dependent kinase. These results suggest that the kinase detected in lysates of mengovirus-infected cells is not the 67-kilodalton kinase. It could be similar to the heme-controlled inhibitor of protein synthesis (for a review, see reference 14) or a hitherto undescribed viral or cellular enzyme.

We also examined the dependence of the mengovirus kinase on dsRNA at different salt concentrations. Salt concentrations of 0.115 M or above inhibited the capacity of the kinase to phosphorylate eIF-2α (Fig. 7B). Furthermore, poly(I) · poly(C) (0.5 μg/ml) had little effect at any of the salt concentrations tested.

The effect of mengovirus kinase on protein synthesis in reticulocyte lysates. The partially purified (by DEAE-cellulose chromatography) kinase was tested for its effect on protein synthesis in a message-dependent reticulocyte lysate

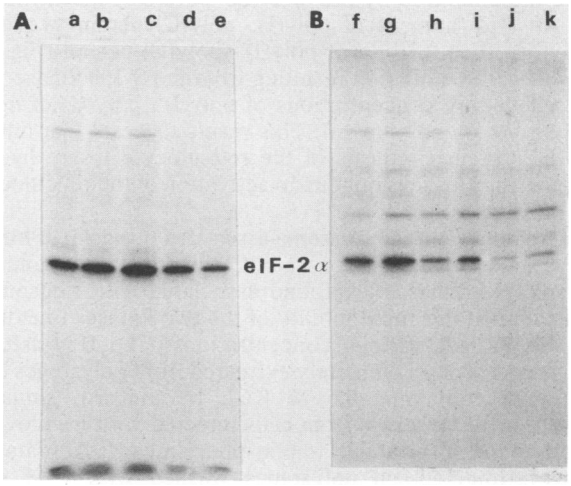

FIG. 7. Effect of poly(I) · poly(C) and KCl on mengovirus kinase activity. (A)The 0.5 M KCl DEAE-cellulose eluate (0.13 μg of protein) from an S-100 fraction of cells infected for 3 h (Fig. 6) was incubated in a final volume of 15 μl with [γ-^{32}P]ATP and 0.7 μg of eIF-2 as described in Materials and Methods. Poly(I) · poly(C) was added in the following concentrations (μg/ml): a, 0; b, 0.05; c, 0.5; d, 5; e, 50. (B) The reaction mixtures were as described above. Lanes: f, h, and j, no poly(I) · poly(C); g, i, and k, 0.5 μg of poly(I) · poly(C) per ml. The KCl concentration (mM) of each reaction mixture was as follows: f and g, 78; h and i, 115; and j and k, 200. The differences in the phosphorylation patterns in A and B result from the use of two different eIF-2 preparations.

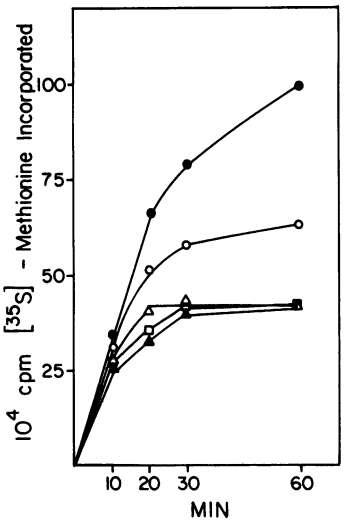

FIG. 8. Effect of mengovirus kinase on protein synthesis in a reticulocyte lysate. The DEAE-cellulose fraction eluting with 0.5 M KCl (Fig. 6) was added to a reticulocyte lysate (25-μl incubation mixture) with brome mosaic virus RNA as the message (see Materials and Methods). Symbols: ●, no additions; ▲, 4 ng of poly(I) · poly(C); □, 1.5 μg of DEAE-cellulose fraction; △, 1.5 ug of DEAE-cellulose fraction and 0.2 μg poly(I) · poly(C); ○, 0.2 μg poly(I) · poly(C).

(see Materials and Methods). The kinase inhibited protein synthesis strongly (Fig. 8). The possibility that the inhibition might be due to the presence in the kinase preparation of dsRNA, which could conceivably activate the reticulocyte dsRNA-dependent kinase, was eliminated by adding high concentrations of poly(I) · poly(C) to the reaction mixture. High concentrations of poly(I) · poly(C) are known to overcome the inhibition by low poly(I) · poly(C) concentrations (7). An 8-μg amount of poly(I) · poly(C) per ml was less inhibitory than 0.16 μg of poly(I) · poly(C) per ml (Fig. 8). However, the inhibition resulting from the S-100 kinase was not relieved by concentrations of poly(I) · poly(C) of up to 40 μg/ml (data not shown). This result suggests that inhibition of protein synthesis in the reticulocyte lysate by the mengovirus kinase is not due to activation of the reticulocyte dsRNA kinase.

Susceptibility of the ribosome-associated mengovirus kinase to extraction by KCl. Because both the mengovirus and the IFN-dsRNA kinases are found associated with ribosomes, we examined the susceptibility of the two kinases to extraction by KCl at various concentrations. The IFN-dsRNA kinase was nearly completely extracted from polysomes by a salt wash containing 0.5 M KCl. In contrast, similarly salt-washed polysomes from cells infected with mengovirus for 5 h retained the ability to phosphorylate eIF-2α (data not shown). Infected-cell polysomes washed with 1 M salt-containing buffers no longer possessed kinase activity.

DISCUSSION

In this report we present evidence for eIF-2α kinase activity associated with mengovirus infection of L929 cells. Kinase activity was detected early (1 h) after infection and was located in the S-100 fraction of the cell for up to 3 h. Later (5 h), kinase activity was also found in the polysome fraction. Since the activity of the kinase was not affected by the presence of actinomycin D, the kinase most likely does not require host cell RNA synthesis for induction.

Since picornavirus-infected cells contain replicative intermediates, we anticipated that the kinase activity seen in lysates of infected cells would be the dsRNA-dependent protein kinase that is present in various amounts in many cells. However, because no phosphorylation of a 67-kilodalton protein characteristic of the dsRNA-dependent kinase was detected in our in vitro assays, no labeling of this protein was found in virus-infected intact cells (6; unpublished data), and no dependence of the kinase on dsRNA for activity was observed, we do not think that the mengovirus kinase is dsI. One could argue that the lack of labeling of the 67-kilodalton protein in vitro is due to its being already activated by endogenous viral dsRNA intermediates present in infected cells. However, the presence of eIF-2α kinase activity in the S-100 fraction rather than associated with ribosomes also speaks against the possibility that we are dealing with the P-1–eIF-2 system. More important, our preliminary results (unpublished) indicate that the mengovirus kinase phosphorylates the α subunit of eIF-2 at a site different from the site phosphorylated by dsI and HCI. Therefore, it appears that the mengovirus kinase may be a hitherto undescribed cellular activity or may be of viral origin. It should be pointed out that there has been a report (20) of increased ribosomal protein S-6 phosphorylation in mengovirus-infected cells, but we have made no attempt to confirm or extend this finding.

Regulation of eIF-2 activity in virus-infected cells has been demonstrated recently in adenovirus- and vaccinia virus-infected cells. In both cases, the P-1–eIF-2 kinase system is induced shortly after infection, presumably by viral dsRNA intermediates. However, in the case of adenovirus-infected cells, activation of the kinase is prevented by a small viral RNA, VAI RNA, that is synthesized during infection (23, 25). In cells infected by mutant adenovirus *dl*331 that cannot synthesize VAI RNA (23, 25), the P-1–eIF-2 kinase is expressed with subsequent cell and viral protein synthesis inhibition. In vaccinia virus-infected cells, P-1–eIF-2 kinase activity is suppressed by a virus-coded enzyme (15, 18, 29). Rosen et al. (19) have demonstrated that mengovirus RNA itself possesses the ability to inhibit reticulocyte dsRNA-dependent kinase activity. It is not known whether mengovirus RNA suppresses this activity in infected cells. As mentioned above, we could not detect induction of the P-1–eIF-2 kinase system either in intact cells or in lysates of infected cells.

The presence in mengovirus-infected cells of kinase activity capable of phosphorylating exogenous eIF-2α is of great interest because eIF-2 phosphorylation may be the means by which this virus inhibits cellular protein synthesis. Indeed, the appearance of kinase activity (at 1 h) correlates with the onset of cellular protein synthesis inhibition (16). Also, there is a strong similarity between the translation inhibitor described by Pensiero and Lucas-Lenard (16) in lysates of mengovirus-infected L cells and the mengovirus kinase. Both inhibitor and kinase are present in the S-100 fraction early in infection and in the ribosome fraction late in infection. However, since the kinase phosphorylates the α subunit of eIF-2 at a site not phosphorylated by either dsI or HCI, little can be said about its possible role in protein synthesis inhibition. The DEAE-cellulose-purified mengovirus kinase inhibited protein synthesis in reticulocyte lysates (Fig. 8), but whether the inhibition is relieved by guanine nucleotide exchange factor or excess eIF-2 remains to be examined. The mengovirus kinase may play a role in phosphorylating viral phosphoproteins in vivo and may only incidently phosphorylate exogenous eIF-2α in vitro. Further

work is necessary to understand the nature and function of the mengovirus kinase.

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