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Biochemistry

Characterization of double-stranded-RNA-activated kinase that phosphorylates α subunit of eukaryotic initiation factor 2 (eIF-2α) in reticulocyte lysates

(purification of eIF-2α kinase/activation in ribosome salt wash/translational control/phosphopeptide mapping of eIF-2α)

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ABSTRACT **Incubation of reticulocyte lysates with low levels of double-stranded (ds) RNA (1–20 ng/ml) activates a cAMP-independent protein kinase (dsI) that phosphorylates the α-subunit (M_r 38,000) of initiation factor 2 (eIF-2) and produces an inhibition of protein chain initiation similar to that caused by heme deficiency. Activation of dsI from its latent precursor takes place on the ribosomes and requires ATP. dsI can also be activated in ribosomal salt washes and in partially purified preparations of the latent precursor of dsI. In all preparations, activation is accompanied by the ds RNA-dependent phosphorylation of a polypeptide doublet that migrates as bands of 67 and 68.5 kilodaltons (67/68.5) in NaDodSO$_4$/acrylamide gels. The rate of phosphorylation of these components in a ribosome salt wash is more rapid than the ds RNA-dependent phosphorylation of eIF-2α. Other polypeptides in the salt wash also undergo ds RNA-dependent phosphorylation, but their significance is not clear. All of these phosphorylations are prevented by high concentrations of poly(I)·poly(C) (20 μg/ml), but not by an antiserum specific for the heme-regulated eIF-2α kinase. Both the latent and activated forms of dsI have been partially purified from a 0.5 M KCl wash of reticulocyte ribosomes. The two species have similar M_rs (≈120,000) and sedimentation coefficients (≈3.75 S), which suggests that activation of dsI probably does not involve extensive changes. By comparison, the heme-regulated eIF-2α kinase has an M_r of ≈160,000 and sediments at ≈6.6 S. However, *in vitro*, dsI and HRI both phosphorylate the same site(s) of eIF-2α. Purified dsI inhibits protein synthesis in hemin-supplemented lysates with the same kinetics induced by the addition of ds RNA; both inhibitions are reversed by eIF-2. dsI that has been activated in the salt wash and then purified does not require ds RNA for expression and no longer displays phosphorylation of the 68.5/67 doublet, which appears to occur only during activation. The data support the view that this component(s) may be the eIF-2α kinase activated by ds RNA.**

The inhibition of protein chain initiation in reticulocyte lysates by low levels (1–10 ng/ml) of double-stranded (ds) RNA can be attributed in part to the activation of a cAMP-independent protein kinase (dsI) that phosphorylates the α-polypeptide (M_r 38,000) of the initiation factor eIF-2 (eIF-2α) (1–5). In contrast to the cAMP-independent heme-regulated eIF-2α kinase (HRI) which can be activated in the postribosomal supernate, activation of dsI occurs on the ribosomes (3–6). The formation of dsI in lysates is rapid and, as in heme deficiency, the inhibition of protein synthesis is characterized by biphasic kinetics with a brief initial period of linear synthesis followed by a sharp shut-off of synthesis (1–4). The phosphorylation of eIF-2α can be directly demonstrated in heme-deficient or ds RNA-treated lysates (7, 8) and is the primary event in both inhibitions (3–5, 9–12), although other initiation components are probably involved in both inhibitory mechanisms (6, 13–17). dsI and HRI are present in normal lysates as inactive precursors. The molecular nature of these latent forms is unknown at present; however, several criteria indicate that the activated forms of the two eIF-2α kinases are different molecular entities. These include differences in sites of activation and sensitivity to hemin control (3, 4) as well as the recent finding that dsI and HRI are immunologically distinct (18). Other differences are reflected in the ^{32}P-labeled phosphoprotein profiles induced in the two inhibited lysates (8). In heme-deficient lysates the phosphorylation of endogenous eIF-2α is accompanied by the phosphorylation of an 80,000 M_r polypeptide that has been identified as endogenous HRI (8). This observation is in accord with studies that indicate that the phosphorylation of isolated HRI is required for its activation (19–21). In ds RNA-treated lysates, no phosphorylation of the 80,000 M_r polypeptide (HRI) takes place, but there is a ds RNA-dependent phosphorylation of a 67,000 M_r polypeptide in addition to endogenous eIF-2α (8). Of interest is the similarity of the ds RNA-induced phosphoprotein profile in lysates to that found in ds RNA-treated extracts prepared from interferon-sensitized nonerythroid cells (22–24). More particularly, it seems evident from several studies that the effects of ds RNA on translation in the lysate system (3–6) are similar in significant respects to those induced in other cells by interferon (6, 25–33).

In previous studies (4, 5), we described some parameters of dsI activation on isolated ribosomes and some properties of the ribosome-bound dsI. We also demonstrated that the particulate dsI could be solubilized by extraction with 0.5 M KCl (4, 5). Subsequently, we showed that dsI can be activated by ds RNA directly in the 0.5 M KCl wash of reticulocyte ribosomes (34); a similar finding has been reported by other investigators (6). In the present study we describe the characterization and partial purification of dsI activated in the ribosomal salt wash.

METHODS AND MATERIALS

Rabbit reticulocytes and reticulocyte lysates were prepared as described (35). Protein synthesis was assayed in 25-μl reaction mixtures containing 12.5 μl of lysate as described (36). Protein kinase assays (20 μl) of the dsI preparations were as described (4) except as noted in the legends. Slab gel electrophoresis (0.1% NaDodSO$_4$/10% acrylamide/0.25% bisacrylamide) and autoradiography were carried out as described (4). Ribosome salt washes were prepared from reticulocyte ribosomes obtained by overnight sedimentation at 120,000 × *g* in a Spinco 42.1 rotor through a 5-ml cushion of 50% glycerol in 20 mM Tris·

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Abbreviations: ds, double-stranded; dsI, ds RNA-activated inhibitor; HRI, heme-regulated inhibitor; eIF-2, eukaryotic initiation factor 2; eIF-2α, 38,000-dalton subunit of eIF-2; cAMP, cyclic AMP; 67/68.5, polypeptide doublet that migrates as bands of 67 and 68.5 kilodaltons; dsI(DC), dsI fraction eluted from DEAE-cellulose; dsI(PC), dsI fraction eluted from phosphocellulose.

HCl, pH 7.5/80 mM KCl/2 mM $Mg(OAc)_2$ (buffer A). The ribosomal pellet was suspended in buffer A and brought to 0.5 M KCl and 38 A_{260} units/ml. After 15 min in ice, the ribosomes were removed by centrifugation and the salt wash was concentrated by $(NH_4)_2SO_4$ precipitation and then dialyzed for 2 hr against 20 mM Tris·HCl, pH 7.5/100 mM KCl/10% glycerol/0.2 mM EDTA (buffer B). After clarification by low-speed centrifugation, the extract was stored at −70°C. dsI was activated directly in the salt wash as described in the text. The crude dsI was partially purified by chromatography on DEAE-cellulose (DC) and phosphocellulose (PC) (see text). Tryptic digestion of [^{32}P]eIF-2α fixed in dry-stained gel slices was carried out by a modification (unpublished results) of the method of Elder *et al.* (37). Ascending chromatography of the tryptic digest on thin-layer cellulose plates (EM Laboratories, Elmsford, NY) was for 4 hr at room temperature in *n*-butanol/pyridine/acetic acid/H_2O, 130:100:20:80 (vol/vol) (unpublished results). Partially purified HRI was prepared from a CM-Sephadex fraction (38) by treatment with *N*-ethylmaleimide (38) as described (4). Preparation of an anti-HRI immune serum was described (18).

Penicillium chrysogenum ds RNA was a gift of Hugh Robertson (Rockefeller University). Highly purified eIF-2 was kindly provided by William C. Merrick (Case–Western Reserve University). [^{14}C]Leucine (355 mCi/mmol; 1 Ci = 3.7 × 10^{10} becquerels) and [γ-^{32}P]ATP (20–30 Ci/mmol) were purchased from New England Nuclear, and trypsin treated with *N*-tosylphenylalanine chloromethyl ketone was obtained from Worthington.

RESULTS

ds RNA-Dependent Phosphoprotein Profile in the Ribosomal Salt Wash. Incubation of a 0.5 M KCl wash of reticulocyte ribosomes with [γ-^{32}P]ATP and low levels of *P. chrysogenum* ds RNA (20 ng/ml) appears to give rise to several ds RNA-dependent ^{32}P-labeled phosphoproteins (Fig. 1). These migrated in $NaDodSO_4$/acrylamide gels (10% acrylamide/0.26% bisacrylamide/0.1% $NaDodSO_4$) with apparent M_rs of 115–120,000; 67,000 and 68,500; 52,000; and 38,000 (eIF-2α) (Fig. 1, tracks 3 and 4). The most significant of these is the 67/68.5 doublet whose phosphorylation accompanied the activation of dsI in all preparations. The phosphorylation of these components was blocked by high concentrations of poly(I)·poly(C) (20 μg/ml) (track 5). An anti-HRI immune serum preparation (18) had no significant effect on any of the ds RNA-dependent phosphorylations (tracks 8 and 9). However, the anti-HRI preparation did block the low level phosphorylation of eIF-2α, which was not ds RNA-dependent (tracks 6 and 7) but was due to a small amount of HRI present in the salt wash preparation. The HRI was removed during purification of the crude dsI preparation.

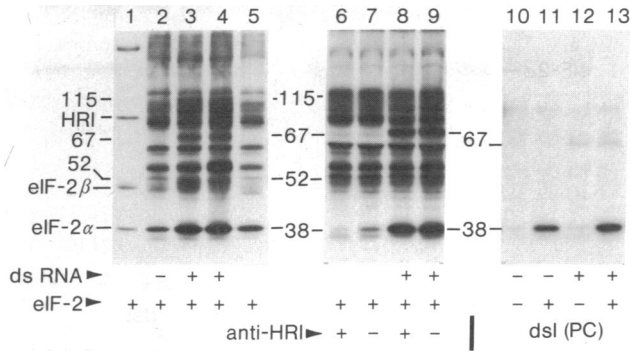

FIG. 1. ^{32}P-labeled phosphoprotein profile of dsI activation in a ribosomal salt wash. Protein kinase assays (20 μl) (4) contained 10 mM Hepes (pH 7.2), 2 mM $Mg(OAc)_2$, 20 mM KCl, and 40 μM [γ-^{32}P]ATP (4 Ci/mmol) and were supplemented where indicated with ds RNA (20 ng/ml), 0.45 μg (2–3 pmol) of eIF-2, 0.5 μl of anti-HRI immune serum (18), poly(I)·poly(C) (20 μg/ml) (track 5), and 6 μg of ribosomal salt wash (tracks 2–9) or 0.7 μg of dsI(PC) (tracks 10–13). One assay (track 1) contained 80 mM KCl and 1.8 μg of *N*-ethylmaleimide-activated HRI. After 15 min at 37°C, the ^{32}P-labeled phosphoprotein profile was analyzed by electrophoresis in $NaDodSO_4$/acrylamide gels (4). The figure is an autoradiogram. Numbers, kilodaltons.

The dsI activated in the 0.5 M KCl wash in response to ds RNA (20 ng/ml) was partially purified by ion-exchange chromatography on DC and PC. Endogenous HRI and eIF-2 were removed by elution from DC at 0.2 M KCl. A dsI fraction was eluted at 0.3 M KCl from DC [dsI(DC)] and was then chromatographed on PC and eluted at 0.2 M KCl; this preparation is designated dsI(PC) (Fig. 1, tracks 10–13). No ds RNA could be detected in dsI(DC) or dsI(PC). In addition, as noted in refs. 3 and 4, after activation dsI activity was expressed without a further requirement for ds RNA (track 11). Nor was expression of dsI activity accompanied by phosphorylation of the 67/68.5 doublet because this is phosphorylated only during dsI activation. Nevertheless, partially purified dsI(PC) produced a small increase in eIF-2α phosphorylation when ds RNA was added (track 13), and this was invariably accompanied by a slight but detectable phosphorylation in the 67/68.5 area (tracks 12 and 13), which indicates that these components are associated with the eIF-2α kinase activity.

The kinetics of phosphorylation of eIF-2α was examined in the salt wash under four sets of conditions (Fig. 2). In the absence of ds RNA (tracks 1–4, Fig. 2*A*), there was a low level phosphorylation of eIF-2α due to contaminating HRI but no phosphorylation of the 67/68.5 polypeptides. The addition of ds RNA (20 ng/ml) (tracks 5–8) produced extensive phosphorylation in 67/68.5 but a limited increase in the amount of [^{32}P]eIF-2α due to low endogenous eIF-2 levels. The full extent of dsI activation was apparent only when the salt wash was supplemented with both ds RNA and eIF-2 (tracks 13–16). The initial kinetics (0–3 min) of phosphorylation of eIF-2α and of 67/68.5 under the various conditions are plotted in Fig. 2*B*. Several points can be made: (*i*) As noted above, the low level of ds RNA-independent eIF-2α kinase activity (Fig. 2*B Left*) is due to HRI. (*ii*) The rate of phosphorylation of eIF-2α was increased 6- to 10-fold when both ds RNA and eIF-2 were added. (*iii*) The ds RNA-dependent phosphorylation of 67/68.5 (Fig. 2*B Center*) is rapid compared to the rate of phosphorylation of eIF-2α and was not affected by the addition of eIF-2. In addition, the ratio of ^{32}P to eIF-2α after 10 min of incubation appears to be greater than 1 (Fig. 2*B Right*); however, because the ^{32}P label in eIF-2α represents the sum of the activated dsI and the endogenous HRI activities, the number of sites on eIF-2α that are phosphorylated by dsI cannot be accurately assigned.

The effects of the salt wash dsI and the dsI(PC) preparations on the kinetics of protein synthesis were examined in normal lysates (Fig. 3). Owing to the presence of some active HRI in the salt wash, and perhaps other inhibitors as well, aliquots of the untreated salt wash were inhibitory (Fig. 3*A*); however, after activation by ds RNA, the salt wash was considerably more inhibitory (Fig. 3*B*). The dsI(PC) preparation (see Fig. 1) inhibited protein synthesis with biphasic kinetics and was efficiently reversed by the delayed addition of purified eIF-2 (Fig. 3*C*).

Effect of Anti-HRI Antiserum on Partially Purified HRI and dsI. The effect of an anti-HRI antiserum (18) on the ability of partially purified dsI and HRI to phosphorylate eIF-2α was

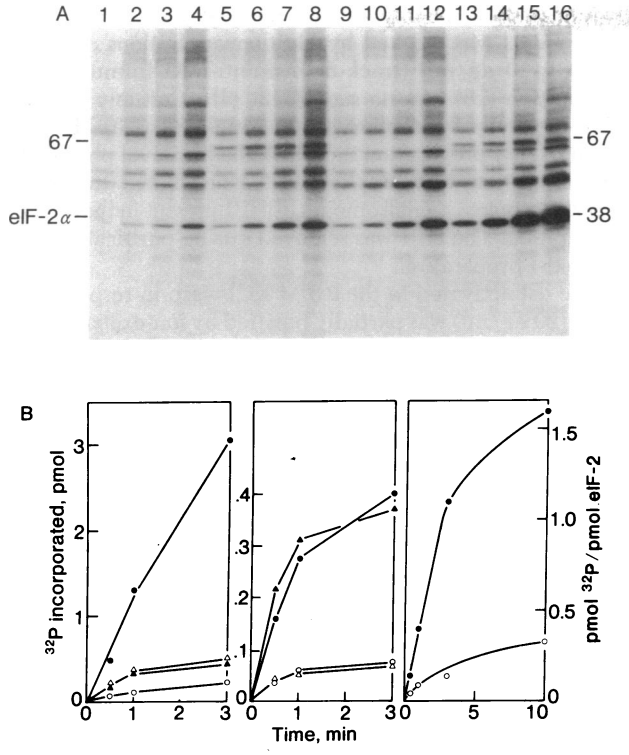

FIG. 2. Kinetics of ds RNA-dependent phoshorylation of eIF-2α and 67/68.5 in a ribosome salt wash. Protein kinase assay (20 μl) (see Fig. 1) were supplemented where indicated with ds RNA (40 ng/ml) and 0.45 μg (2–3 pmol) of eIF-2. All assays contained 6 μg of salt wash proteins. For each set of conditions, assays were incubated for 0.5, 1, 3, and 10 min at 37°C. (*A*) Proteins were separated by electrophoresis in NaDodSO$_4$/acrylamide as described (4). Tracks: 1–4, no additions; 5–8, plus ds RNA; 9–12, plus eIF-2; 13–16, plus ds RNA and eIF-2. After the gel was stained and dried, the eIF-2α and the 67/68.5 bands were cut out, dissolved in 1 ml of H_2O_2 (16 hr at 40°C), and assayed for radioactivity. (*B*) Plot of data in *A*. (*Left*) eIF-2α. ○, −eIF-2; ▲, +ds RNA; △, +eIF-2; ●, +ds RNA and eIF-2. (*Center*) The 67/68.5 doublet. Symbols are as in *Left*. (*Right*) ^{32}P-to-eIF-2α ratio. ●, +ds RNA; ○, −ds RNA.

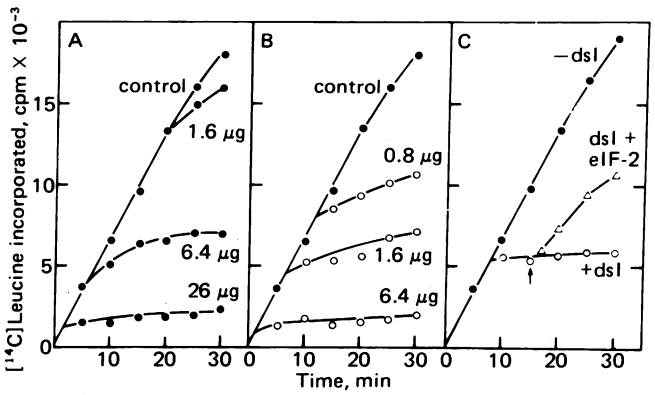

FIG. 3. Effect of crude and partially purified dsI on the kinetics of protein synthesis. Protein synthesis assays (25 μl) were carried out as described (36). Control salt wash (*A*) and salt wash preincubated for 30 min at 30°C with *P. chrysogenum* ds RNA at 20 ng/ml to activate dsI (4) (*B*) were added in the indicated amounts. In *C*, 0.55 μg of partially purified dsI(PC) (see Fig. 1) was added at 0 min where indicated, and 2 μg of eIF-2 (≈12 pmol) was added at 15 min as indicated (arrow). Assays in *A* and *B* all contained 10 μg/ml of poly(I)·poly(C) (4). At the indicated intervals, 3-μl aliquots were assayed for protein synthesis (4, 36).

examined (Fig. 4). Both of the eIF-2α kinase preparations utilized in this experiment contained cAMP-independent eIF-2β kinases; these activities are not inhibitory (4, 39, 40) and are not affected by anti-HRI antiserum (18) (tracks 3, 4, 8, and 10). The use of relatively high levels of HRI (tracks 1–5) and relatively low levels of dsI (tracks 6–10) emphasizes the immunospecificity of the anti-HRI antiserum (tracks 3, 5, 8, and 9) compared to the lack of immunological activity in a control serum (tracks 2, 4, 7, and 9). At the two dilutions of anti-HRI antiserum utilized in these assays, the phosphorylations of both HRI (M_r 80,000) and eIF-2α were largely abolished (tracks 3 and 5), whereas the phosphorylation of eIF-2α by dsI was not affected (tracks 8 and 10).

Comparison of Tryptic Phosphopeptides of eIF-2α After Phosphorylation by HRI and dsI. To compare the site specificity of phosphorylation of eIF-2α by HRI and dsI, we carried out protein kinase assays with partially purified dsI and HRI, as well as with the corresponding eIF-2α kinase activities in the salt wash. Each kinase preparation was assayed with [γ-^{32}P]ATP and purified eIF-2 for 5 and 30 min and the proteins were then separated by NaDodSO$_4$/acrylamide gel electrophoresis (Fig. 5*A*). The [^{32}P]eIF-2α bands from the stained dried gels were excised and digested overnight with *N*-tosylphenylalanine chloromethyl ketone-treated trypsin (Worthington) (37), and the ^{32}P-labeled phosphopeptides were separated on thin-layer cellulose plates (EM Laboratories) by ascending chromatography (Fig. 5*B*) (41). All of the digestions yielded similar ^{32}P-labeled phosphopeptide patterns. This was confirmed by a densitometric scan of each profile in the autoradiogram, all of which were remarkably similar (data not shown). Similar data were obtained when the tryptic digests were separated by electrophoresis at two different pHs; in each method of separation, the ^{32}P-labeled phosphopeptide distribution was the same for all assays. These data do not clarify the number of sites that are phosphorylated by dsI or HRI, which may be no more

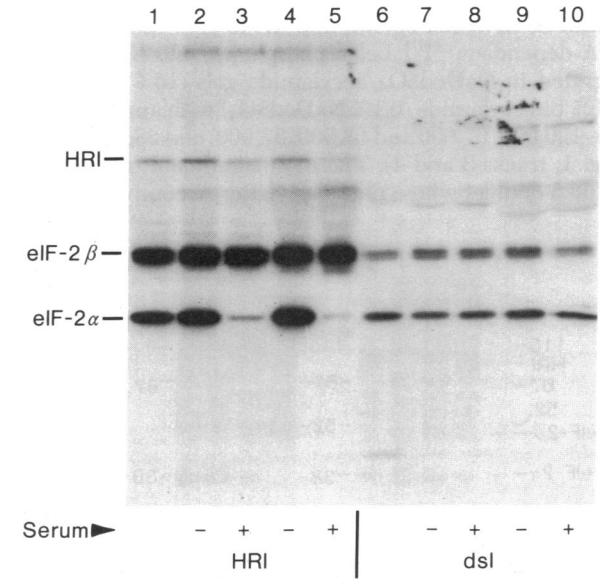

FIG. 4. Effect of anti-HRI antiserum on partially purified dsI and HRI activity. Protein kinase assays (20 μl) contained 10 mM Hepes (pH 7.2), 2 mM Mg(OAc)$_2$, 60 mM KCl, and 100 μM [γ-^{32}P]ATP (3 Ci/mmol), and were supplemented where indicated with 1.8 μg of *N*-ethylmaleimide-activated HRI (38) (tracks 1–5) or 1.1 μg of a dsI preparation activated in the salt wash and chromatographed on DC (tracks 6–10) (see text). Both preparations also contained eIF-2β kinase activities (4). All assays contained 0.3 μg (≈2 pmol) of eIF-2. Serum additions were as follows: 0.2 μl of control serum (tracks 2 and 7) or antiserum (tracks 3 and 8); 0.7 μl of control serum (tracks 4 and 9) or antiserum (tracks 5 and 10) (18). After 5 min at 30°C assay samples were subjected to electrophoresis in a NaDodSO$_4$/acrylamide gel (4). The figure is an autoradiogram.

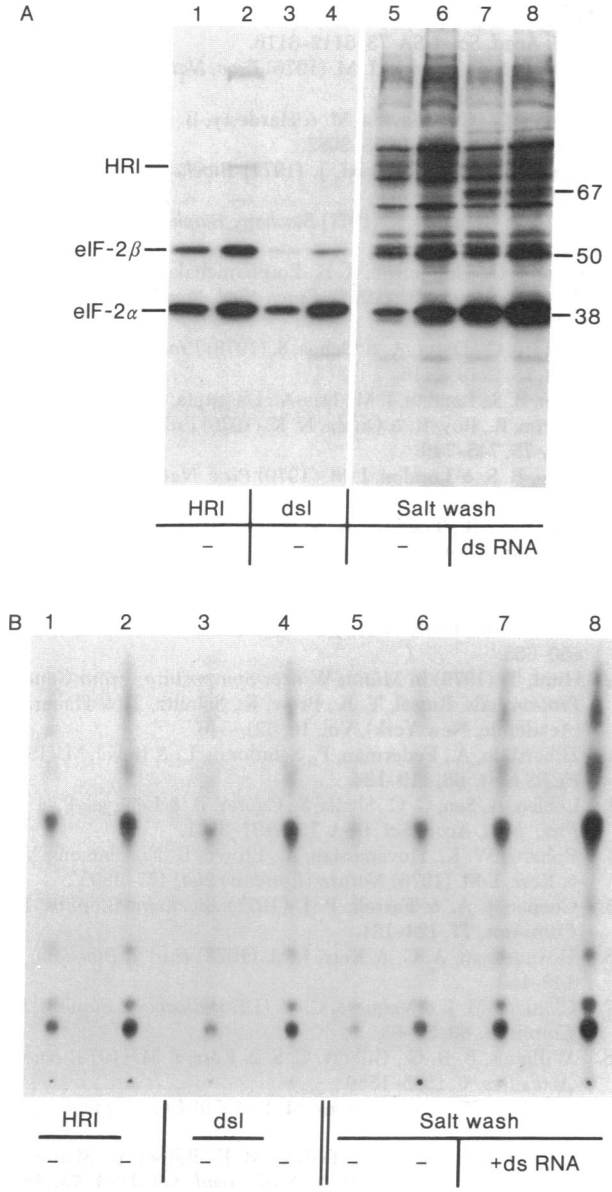

FIG. 5. Trypsin digestion of [^{32}P]eIF-2α labeled by crude and partially purified HRI and dsI. Protein kinase assays (20 μl) (see Fig. 4) were supplemented where indicated with 1.8 μg of *N*-ethylmaleimide-activated HRI (38) (tracks 1 and 2), 1.1 μg of a dsI(DC) preparation (see Fig. 4) (tracks 3 and 4), or 6 μg of ribosomal salt wash (tracks 5–8). *P. chrysogenum* ds RNA (20 ng/ml) was added to assays 7 and 8. After 5 min at 30°C (tracks 1, 3, 5, and 7) or 30 min at 30°C (tracks 2, 4, 6, and 8) proteins were separated by electrophoresis (*A*). The dried stained [^{32}P]eIF-2α band was cut out, digested with *N*-tosylphenylalanine chloromethyl ketone-treated trypsin (37), and chromatographed on thin-layer cellulose plates (41). (*B*) Autoradiogram of the chromatographic separation. Each numbered track represents the tryptic digest of the [^{32}P]eIF-2α of the corresponding track in *A*.

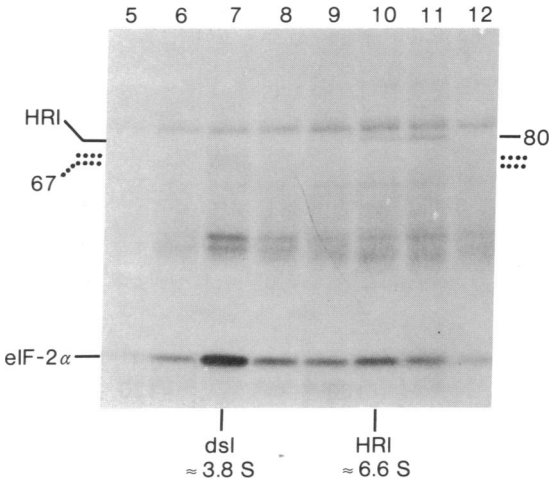

FIG. 6. Separation of dsI and HRI by gradient centrifugation. About 50 μl of a dsI(DC) preparation containing low levels of HRI was loaded on a 4.8-ml glycerol gradient (10–40%) (in 25 mM Tris·HCl, pH 7.5/80 mM KCl/0.2 mM EDTA) and centrifuged at 45,000 rpm for 20 hr at 4°C in a Beckman Spinco SW50.1 rotor; 18 0.25-ml fractions were collected and assayed directly for eIF-2α kinase activity in 20-μl assays containing 10 mM Hepes (pH 7.2), 2 mM $Mg(OAc)_2$, 60 mM KCl, 30 μM [γ-^{32}P]ATP (4 Ci/mmol), 0.2 μg of eIF-2 (≈1.4 pmol), ds RNA (20 ng/ml), and 10 μl of each fraction in the gradient. After 30 min at 37°C, the proteins were separated by electrophoresis in a $NaDodSO_4$/acrylamide gel (4). The figure is an autoradiogram of the incubations with gradient fractions 5–12.

than one or two (3, 4, 39, 42). However, the data do suggest two fundamental conclusions. (*i*) Partially purified HRI and dsI phosphorylate the same site(s) on eIF-2α. (*ii*) The pattern of phosphorylation of eIF-2α by dsI or HRI is the same at early and late time intervals, which supports a model of specific phosphorylation. Recent extensive phosphopeptide mapping studies using several proteases have confirmed these findings and, more significantly, have demonstrated that the site specificity of eIF-2α phosphorylation by partially purified dsI or HRI is the same for endogenous eIF-2α in heme-deficient or ds RNA-treated lysates (unpublished results). In addition, a recent study (33) indicates that HRI isolated from lysates has the same site specificity toward eIF-2α *in vitro* as a ds RNA-dependent eIF-2α kinase contained in the salt wash of interferon-treated human amnion U cells.

Sedimentation of dsI and HRI in a Glycerol Gradient. In order to compare the sedimentation properties of dsI and HRI, dsI was activated in the presence of ds RNA and unlabeled ATP in a ribosome salt wash that also contained low levels of HRI. The mixture was then chromatographed on DC. A fraction that eluted at 0.2 M KCl (in buffer B) contained both dsI and HRI activity. The two protein kinases were then separated by centrifugation in a 10–40% glycerol gradient (Fig. 6), yielding sedimentation coefficients of ≈3.7 S for dsI (track 7) and ≈6.6 S for HRI (track 10). When eIF-2α kinase activity was monitored across the gradient in the presence of ds RNA, there was little or no phosphorylation of the 67/68.5 doublet as expected (track 7). However, the 80,000-dalton component of HRI could be clearly distinguished (track 10). We have recently found that both the active and latent forms of partially purified dsI have similar sedimentation coefficients [≈3.6–3.8 S (unpublished results)].

Sizing of dsI. A preparation of the latent inactive form of dsI was partially purified from the ribosomal salt wash (unpublished results) and activated in the presence of ds RNA, eIF-2, and [γ-^{32}P]ATP. The incubation mixture was then filtered through Sephadex G-200; fractions were collected, and each fraction was separated by electrophoresis in $NaDodSO_4$/acrylamide gels (Fig. 7). [^{32}P]eIF-2 labeled in the α-subunit (M_r 38,000) eluted at an approximate M_r of 150–160,000. dsI activity eluted at an approximate M_r of 120,000 and was coincident with a ds [^{32}P]RNA-dependent polypeptide doublet of M_rs 67,000 and 68,500 (Fig. 7). The same molecular-weight profiles in G-200 were obtained with dsI(PC), which was activated in the salt wash and then chromatographed (see Fig. 1), and with the latent form of dsI (data not shown); these results indicate that the inactive and active forms of dsI have similar molecular weights.

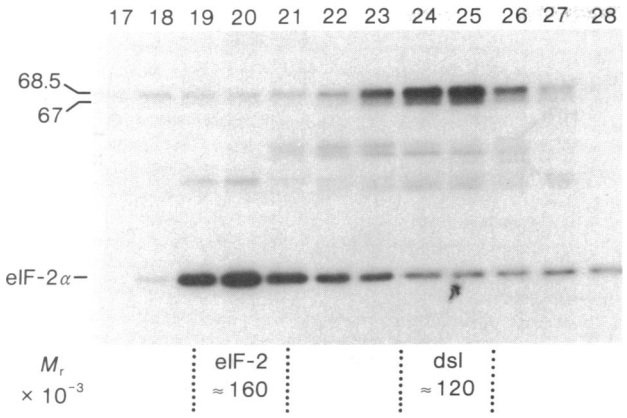

FIG. 7. Filtration of dsI in Sephadex G-200. A protein kinase assay (200 μl) contained 10 mM Hepes (pH 7.2), 2 mM $Mg(OAc)_2$, 20 mM KCl, 35 μM [γ-^{32}P]ATP (5 Ci/mmol), 6.6 μg of eIF-2 (≈44 pmol), *P. chrysogenum* ds RNA (20 ng/ml), and 11 μg of a partially purified latent dsI preparation (unpublished results). Incubation was for 10 min at 37°C. The reaction mixture was chilled, filtered through a Sephadex G-200 (superfine) column (1 × 25 cm), and collected in 0.36-ml fractions in the cold. Each fraction was supplemented with carrier ovalbumin (10 μg) and concentrated by precipitation with 10% trichloroacetic acid. The precipitates were dissolved in $NaDodSO_4$-containing dissociation buffer (4) and subjected to electrophoresis in a $NaDodSO_4$/acrylamide gel (4). The figure is an autoradiogram of fractions 17–28.

The molecular nature of dsI is still unclear. Efforts to purify the ds RNA-activated eIF-2α kinase from reticulocyte lysates or extracts of interferon-treated cells (29–31) indicate that the activity from all sources copurifies with the M_r 67,000 component(s), which supports the original suggestion by Farrell *et al.* (3) that this polypeptide(s) may be the protein kinase. In the present studies, we have repeatedly observed that the activation of dsI in the ribosomal salt wash, or with partially purified latent dsI, is associated with the phosphorylation of the 67/68.5 polypeptide doublet in which the M_r 68,500 polypeptide contains more ^{32}P label than the M_r 67,000 component (see Fig. 7). The relationship of these two polypeptides is not clear. However, it is apparent from this and other studies that the lysate dsI and the ds RNA-activated eIF-2α kinase in interferon-treated cells are similar and have the same function in the regulation of protein synthesis.

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