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Purification and properties of the double-stranded RNA-activated eukaryotic initiation factor 2 kinase from rabbit reticulocytes

(polypeptide chain initiation/protein phosphorylation/ternary complex formation/translational control by double-stranded RNA)

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ABSTRACT **The double-stranded RNA (dsRNA)-activated protein kinase (DAI) that phosphorylates the α subunit of the eukaryotic initiation factor eIF-2 and inhibits chain initiation has been isolated from rabbit reticulocyte lysates. The nonactivated enzyme or the enzyme partially activated by incubation with low levels of dsRNA (pro-DAI) could be purified only to a slight extent. However, the enzyme that was fully activated by incubation with both dsRNA and ATP was purified to near homogeneity. Active DAI is a phosphoprotein with an apparent subunit mass of 68,000 daltons. It can phosphorylate histone as well as the α subunit of eIF-2. Our results suggest that, after interaction with dsRNA, the enzyme phosphorylates itself and is thereby activated to phosphorylate α eIF-2 and histone.**

Reticulocyte lysates contain two protein kinases that, upon activation, phosphorylate the small, α, subunit of the eukaryotic initiation factor eIF-2 and inhibit chain initiation (1, 2). One of these enzymes (heme-controlled inhibitor, HCI) is activated by heme deficiency, the other (double-stranded RNA-activated inhibitor, DAI) is activated by low concentrations of double-stranded RNA (dsRNA). Both enzymes phosphorylate the same site(s) of eIF-2 α (3). HCI and DAI differ in a number of ways: (*i*) HCI is present in the postribosomal supernatant, whereas DAI is ribosome associated (1, 2, 4); (*ii*) the subunit molecular weight of HCI [about 90,000 (5)] is higher than that of DAI [about 68,000 (this paper]; (*iii*) HCI is activated by *N*-ethylmaleimide (MalNEt) (6) but DAI is inhibited (ref. 7; this paper); (*iv*) HCI specifically phosphorylates α eIF-2 (1, 8–10) but DAI can also phosphorylate histone (refs. 11 and 12; this paper); and (*v*) HCI and DAI do not crossreact immunologically (13). Both HCI and DAI are present in reticulocytes, but unlike HCI, DAI is induced in other cells by interferon (11, 12, 14).

There is little doubt that the translational inhibition observed after activation of HCI or DAI is related to the phosphorylation of eIF-2 α (1, 15, 16). This effect is highly specific. Thus, phosphorylation of the eIF-2 β subunit by a cAMP-independent protein kinase (casein kinase) from reticulocyte lysates has no effect on translation (2, 17, 18). The way in which phosphorylation of the eIF-2 α subunit inhibits chain initiation has not been fully elucidated, but this subunit is known to interfere with stimulation of eIF-2 activity by the eIF-2-stimulating protein (ESP) (19–23), whereas phosphorylation of the eIF-2 β subunit does not (21).

We have been able to purify reticulocyte pro-DAI only partially, but have purified preactivated DAI to near homogeneity as a phosphoprotein with a subunit mass of approximately 68,000 daltons. Our results suggest that, after interaction with dsRNA, DAI undergoes self-phosphorylation and is thereby activated to phosphorylate the α subunit of eIF-2 as well as histone.

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MATERIALS AND METHODS

Ternary Complex Formation Assay. Active DAI inhibits ternary complex formation (4, 7), and this reaction was used routinely for DAI assay. The assay was conducted in three stages as follows:

Stage 1. First step of DAI activation: Incubation with dsRNA. Unless otherwise specified, samples (30 μl) containing 20 mM Hepes buffer at pH 7.6, 100 mM KCl, 1.5 mM $Mg(OAc)_2$, 1 mM dithiothreitol, DAI as indicated in the legends, and dsRNA [unless otherwise noted, poly(I)·poly(C) at 0.16 μg/ml], were incubated for 5 min at 30°C. Controls without dsRNA were run occasionally.

Stage 2. Second step (phosphorylation) of DAI activation and phosphorylation of eIF-2 α subunit. Samples (40 μl) containing 25 mM Hepes buffer at pH 7.6, 120 mM KCl, 2.5 mM $Mg(OAc)_2$, 1.25 mM dithiothreitol, about 4 pmol of eIF-2 CM-350 (or as specified in the legends), 0.05–0.125 mM ATP, and a suitable aliquot of the incubated stage 1 sample, were incubated for 5 min at 30°C.

Stage 3. Ternary complex formation. The incubated stage 2 samples were supplemented with 1 pmol of [^{35}S]Met-tRNA$_i$ (about 160,000 cpm), 0.2 mM GTP, and 16 μg of CM-200 ESP, unless otherwise noted. The volume was made up to 50 μl and the samples were incubated for 5 min at 30°C. The reaction was stopped by addition of 3 ml of ice-cold buffer [20 mM Tris·HCl, pH 7.6/100 mM KCl/2 mM $Mg(OAc)_2$]. The samples were filtered through nitrocellulose membranes and the retained radioactivity was measured. eIF-2 and ESP were prepared as described by de Haro and Ochoa (21). The two factors were eluted together from the DEAE-cellulose column by raising the concentration of KCl to 200 mM and were separated from each other by chromatography on carboxymethyl-Sephadex. ESP was eluted with 200 mM KCl and eIF-2 with 350 mM KCl. These preparations are referred to as ESP CM-200 and eIF-2 CM-350. For some experiments the eIF-2 was further purified by chromatography on phosphocellulose (preparation eIF-2 PC) as described (21). As judged by polyacrylamide/$NaDodSO_4$ gel electrophoresis, eIF-2 CM-350 was 20–25% pure; eIF-2 PC was 70–75% pure.

Other Assays. Occasionally DAI was assayed by measuring the phosphorylation of the eIF-2 α subunit or of histone IIa, after incubation with [γ-^{32}P]ATP. The incubation samples (25 μl), containing 20 mM Hepes buffer at pH 7.6, 100 mM KCl, 3.5 mM $Mg(OAc)_2$, 0.033 mM [^{32}P]ATP (2000 cpm/pmol), about 7 pmol of eIF-2 PC, or 25 μg of histone IIa, and an ap-

Abbreviations: HCI, heme-controlled translational inhibitor; DAI, double-stranded RNA-activated translational inhibitor; dsRNA, double-stranded RNA; MalNEt, *N*-ethylmaleimide; eIF-2, eukaryotic initiation factor 2; ESP, eIF-2-stimulating protein.

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propriate amount of preactivated DAI, were incubated for 7 min at 30°C. After incubation, duplicate samples were subjected to polyacrylamide/NaDodSO$_4$ gel electrophoresis and stained with Coomassie blue. An autoradiogram was obtained from one of the gel slabs. The eIF-2 α band, or the histone band, was cut out from the other slab, and the gel section was dissolved in H_2O_2 (3 hr, 95°C) and its radioactivity was measured.

Protein was generally determined by the Lowry method (24), but with very dilute protein solutions, the more sensitive Bradford procedure (25) was used. Bovine serum albumin was used as the standard in both cases.

Polyacrylamide/NaDodSO$_4$ Gel Electrophoresis and Autoradiography. Polyacrylamide/NaDodSO$_4$ gel electrophoresis was carried out essentially by Laemmli's procedure (26) in 0.1% NaDodSO$_4$/10% acrylamide/0.1% *N,N'*-methylenebisacrylamide, pH 7.6, for 4 hr at 3.5 mA. Gels were stained with 0.2% Coomassie blue or by the more sensitive silver reduction method (27). For autoradiography the gels were dried, placed on Kodak X-Omat RP x-ray film, and exposed for an appropriate length of time.

Preparation of DAI DE-80 Fraction. DE-80 fraction was prepared from the ribosomal salt wash of reticulocyte lysates. All operations were conducted at 0–2°C. One liter of rabbit reticulocytes (Pel-Freez) yielded about 400 ml of lysate. Ribosomal salt wash (640 mg of protein), prepared as described (19), was dialyzed overnight against buffer A [20 mM Hepes buffer, pH 7.6/0.1 mM EDTA/1 mM dithiothreitol, 5% (vol/vol) glycerol] containing 80 mM KCl. The dialyzed solution (20 ml) was applied to a DEAE-cellulose (Whatman DE-52) column (1.5 × 16 cm) equilibrated with buffer A containing 80 mM KCl and washed with the same buffer until the A_{280} decreased to below 0.2 (21). The bulk of the DAI activity was in the wash (22 ml, 550 mg of protein). This fraction was used for some experiments after precipitation with ammonium sulfate at 70% saturation, followed by dialysis against buffer A.

Preparation of Sepharose-Histone Column. Fifty grams of Sepharose 4B was washed with water on a Buchner funnel and suspended in 100 ml of 2.0 M K_2CO_3 (pH 11.0) with mechanical stirring in the cold room. Ten milliliters of cyanogen bromide dissolved in acetonitrile (1.5 g/ml) was then added with continuous stirring for 90 sec. The activated Sepharose was washed exhaustively with cold water and added to 150 mg of histone (Sigma IIa from calf thymus) suspended in 30 ml of 0.2 M $NaHCO_3$ containing 0.5 M KCl and stirred overnight at 0°C.

RESULTS

DAI Activation. One polypeptide (M_r about 68,000) is heavily labeled when DAI DE-80 is activated with dsRNA in the presence of [γ-^{32}P]ATP (Fig. 1, cf. tracks 1 and 3). The faintly labeled peptide in track 3 (M_r about 100,000) is a contaminant that is removed in the last step of purification of preactivated DAI (see below). In the presence of dsRNA and eIF-2 an additional peptide (M_r about 38,000) is labeled. This is the small α subunit of eIF-2 (Fig. 3, cf. tracks 4 and 6). Neither the M_r 68,000 nor the M_r 38,000 peptide is labeled when DAI is previously incubated with MalNEt (Fig. 1, tracks 2 and 5). Histone IIa is also strongly phosphorylated in the presence of dsRNA and less so in its absence or when the DAI in the dsRNA-containing sample has been preincubated with MalNEt (Fig. 1, tracks 7–9). Note phosphorylation of the M_r 68,000 peptide in track 9. The relatively intense phosphorylation in tracks 7 and 8 is due to the presence of non-dsRNA-dependent histone kinase(s) in DAI DE-80.

The optimal concentration of poly(I)·poly(C) for activation of DAI DE-80 varied with different batches from about 0.2 to 1 μg/ml. With reovirus RNA the optimal concentration was around 0.05 μg/ml. There was no activation at 0.001 or at 10 μg/ml. The reovirus RNA was the kind gift of A. Shatkin.

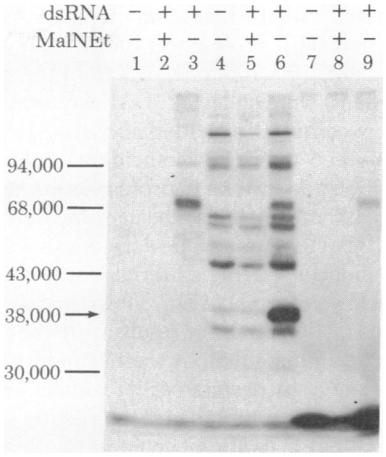

FIG. 1. Phosphorylation of 68-kilodalton polypeptide, eIF-2 α subunit, and histone, upon activation of DAI and inhibition of DAI activation by MalNEt. The incubations were conducted in two steps. In step I the samples (25 μl) contained 20 mM Hepes buffer at pH 7.4, 100 mM KCl, 1.5 mM Mg(OAc)$_2$, 1 mM dithiothreitol, and 7.2 μg of pro-DAI DE-80 [specific activity after activation, about 75 units (see Table 1) per mg of protein], without or with poly(I)·poly(C) (1 μg/ml), and either without further additions (tracks 1–3) or with addition of either 9 pmol (approximately) of eIF-2 CM-350 (tracks 4–6) or 20 μg of histone IIa (tracks 7–9). The samples were incubated for 5 min at 30°C. Duplicates of each of three samples (no substrate, eIF-2, histone) that were to receive poly(I)·poly(C) were prepared by preincubating DAI (5 min, 30°C) with MalNEt (10 mM), followed by neutralization of the unreacted MalNEt with dithiothreitol (20 mM), addition of the remaining components, and incubation as above. In step II, the samples were supplemented with [γ-^{32}P]ATP (0.05 mM, specific radioactivity, 3000 cpm/pmol) and incubated for 7 min at 30°C. Polyacrylamide/NaDodSO$_4$ gel electrophoresis and autoradiography were performed as described in the text.

DAI, like HCI (19–23), blocks the eIF-2–ESP interaction. Fig. 2 shows the time course of ternary complex formation and the pronounced stimulation by ESP which, by itself, binds no Met-tRNA$_i$. In the presence of DAI and ATP the stimulation of complex formation by ESP is markedly reduced.

Purification of Pro-DAI. DAI DE-80 (20 ml, 530 mg of protein) was dialyzed against a solution containing 20 mM potassium phosphate buffer at pH 6.7, 1 mM dithiothreitol, 25 mM KCl, and 10% (vol/vol) glycerol. The dialyzed solution (19 ml) was applied to a column (1.3 × 25 cm) of phosphocellulose (Whatman P-11) equilibrated with the same solution. The

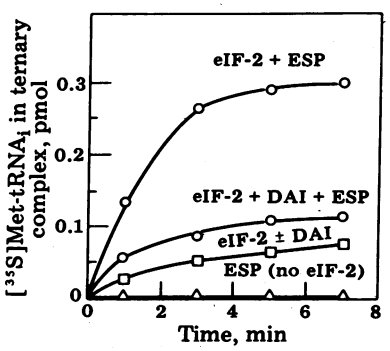

FIG. 2. Time course of ternary complex formation with and without ESP and DAI + ATP. The standard assay was used. Reactants were used in the following amounts: eIF-2 (CM-350), about 4.5 pmol; ESP (CM-200), 16 μg; preactivated DAI DE-80, 16 μg.

column was washed with the potassium phosphate solution until the A_{280} was below 0.01. Protein was then eluted with 500 ml of a linear gradient (100–700 mM) of KCl in the same buffer and fractions (6 ml) were collected. DAI was assayed by inhibition of ternary complex formation and by histone IIa phosphorylation. As seen in Fig. 3, coincident peaks of ternary complex inhibition and histone phosphorylation activity were eluted at about 300 mM KCl just ahead of the bulk of the protein. The specific activity of the peak fractions was 3–4 times higher than that of the starting material, but the yield of units (about 7%) was low. When DAI DE-80 was preincubated with dsRNA prior to fractionation, the results were the same except that no further addition of dsRNA was needed for assay of the fractions. This suggests [despite earlier indications that activation of DAI requires simultaneous interaction with dsRNA and ATP (1, 2)] that activation occurs in two separate steps: interaction with dsRNA followed by interaction with ATP. We have some indications that in the first step dsRNA binds to DAI. When one adds a small amount of ^{3}H-labeled poly(I)·poly(C) (Miles) to DAI DE-80 and fractionates the enzyme on phosphocellulose as above, about 6% of the radioactivity is eluted as a sharp peak together with ternary complex inhibitory activity (Fig. 4A) ahead of the bulk of the protein. The balance of the radioactivity remains bound to the column even after elution with 1 M KCl. This may be due to binding of the polymer to basic protein(s) in DAI DE-80 because, in control experiments without DAI, no poly(I)·poly(C) radioactivity was retained. When DAI was inactivated with MalNEt before mixing with [^{3}H]poly(I)·poly(C) (Fig. 4B) or when [^{3}H]poly(C) (Miles) was substituted for [^{3}H]poly(I)·poly(C) (Fig. 4C) no radioactivity was eluted in the DAI region. This suggests that binding of dsRNA is the first step in DAI activation and that MalNEt prevents activation by blocking dsRNA binding.

After full activation with dsRNA and ATP, DAI becomes very acidic and can no longer be chromatographed on phosphocellulose. The enzyme now binds strongly to DEAE-cellulose, from which it can be eluted, with considerable purification, at high KCl concentrations.

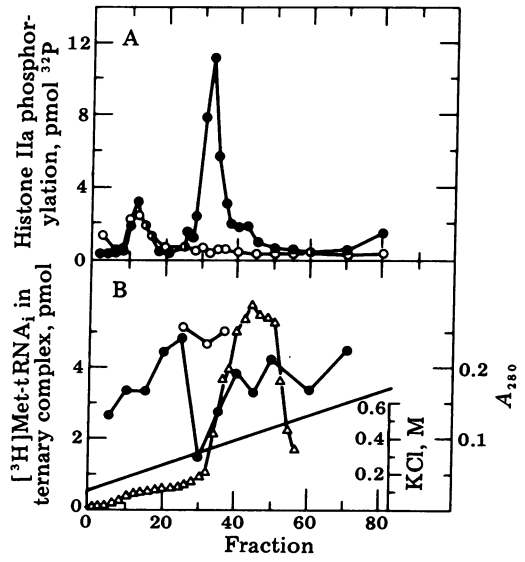

FIG. 3. Phosphocellulose chromatography of pro-DAI. DE-80 fraction was chromatographed on phosphocellulose as described and assayed for inhibition of ternary complex formation (B) or phosphorylation of histone IIa (A) with (●) or without (○) dsRNA activation but after preincubation with ATP in all cases. Reovirus RNA was used for activation. △, A_{280}.

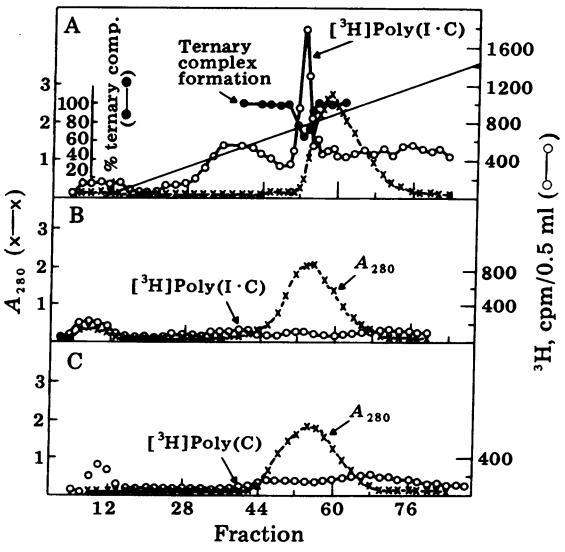

FIG. 4. Binding of [^{3}H]poly(I)·poly(C) by DAI. DAI DE-80 (21 mg of protein) was incubated for 10 min at 30°C, in a final volume of 7.5 ml, either with [^{3}H]poly(I)·poly(C) (223,000 cpm, 38,000 cpm/μg) without (A) or with (B) prior treatment with MalNEt (see legend to Fig. 1) or with [^{3}H]poly(C) (201,000 cpm, 49,624 cpm/μg) (C). The samples were then chromatographed on phosphocellulose as described in the text for purification of pro-DAI. The A_{280} and radioactivity were measured in all gradient fractions from each experiment. The mid fractions in the experiment corresponding to panel A were also assayed for inhibition of ternary complex formation.

Purification and Properties of Preactivated DAI. Activated DAI DE-80 (specific activity in ternary complex inhibition assay, about 70 units/mg) yielded 30% of the enzyme, at specific activity about 170,000, on chromatography on DEAE-cellulose. This material was eluted with KCl-containing buffer A when the KCl concentration was raised from 0.4 to 1.0 M. This enzyme was not homogeneous. Virtually homogeneous DAI was obtained by using a two-step procedure involving DEAE-cellulose chromatography followed by affinity chromatography on Sepharose-histone as described below.

DAI DE-80 (37 ml, 500 mg of protein) was activated by incubation for 10 min at 30°C in a reaction mixture (100 ml) containing 20 mM Hepes buffer at pH 7.6, 2 mM $Mg(OAc)_2$, 30 mM KCl, 1 mM dithiothreitol, poly(I)·poly(C) at 0.6 mg/ml, 0.17 mM ATP, and 20% (vol/vol) glycerol. After incubation, the mixture was cooled in ice and applied to a column (0.9 × 12 cm) of DEAE-cellulose (Whatman DE-52) equilibrated with buffer A containing 80 mM KCl. All operations were conducted at 0–2°C. The column was washed with the 80 mM KCl-containing buffer A and then with buffer A containing 200 mM KCl until the A_{280} of the effluent was below 0.02. More protein was then eluted with buffer A containing 800 mM KCl. The protein-containing fractions were pooled and the solution was diluted with buffer A to make the KCl concentration 500 mM. The DE-800 fraction (5 ml) was applied to a Sepharose-histone column (0.4 × 5 cm), previously equilibrated with buffer A containing 500 mM KCl, and the column was washed with the same buffer until the A_{280} was below 0.01. DAI was then eluted with buffer A containing 1.0 M KCl. The peak activity fractions were pooled to yield 3.6 ml of solution containing 5 μg of protein per ml. We found highly purified DAI to be extremely unstable; it did not withstand storage for more than a few hours under a variety of conditions.

A summary of the purification procedure is given in Table 1. When assayed by ternary complex inhibition, the Sepharose-histone DAI was purified 5000-fold from the DE-80

Table 1. Purification of preactivated DAI

Step	Protein, mg	Ternary complex inhibition assay Units*	Sp. act.	Histone phosphorylation assay Units†	Sp. act.
DE-80 before rechromatography	500	38,000	76	160,000	320
DEAE-cellulose rechromatography, DE-200	426	2,600	6		
DEAE-cellulose rechromatography, DE-800	0.8	11,000	13,750	39,000	48,750
Sepharose-histone chromatography	0.02	7,600	380,000	9,000	450,000

Sp. act., specific activity: units/mg protein. Protein was determined by the Bradford procedure (25).

* One unit, amount of enzyme causing 50% inhibition of ternary complex formation under standard assay conditions.

† One unit, amount of enzyme causing the transfer of 1 pmol of ^{32}P from [γ-^{32}P]ATP to histone IIa under standard assay conditions.

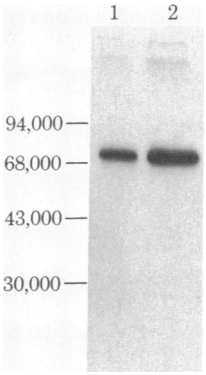

FIG. 6. Autoradiogram of [^{32}P]DAI. Sepharose-histone DAI was prepared as described in the text from DAI DE-80 (84 mg of protein) that had been activated, in a final volume of 20 ml, with poly(I)·poly(C) at 0.6 μg/ml and 50 μM [γ-^{32}P]ATP (1200 cpm/pmol). The Sepharose-histone fraction was dialyzed and concentrated by partial lyophilization prior to polyacrylamide/NaDodSO$_4$ gel electrophoresis and autoradiography. The radioactivity of the protein applied to the gel was 3600 cpm in track 1 and 7200 cpm in track 2. The migration positions of molecular weight markers are shown at the left.

fraction. When assayed by histone IIa phosphorylation the degree of purification appeared to be much less (about 1400-fold). It should be noted, however, that the DE-80 fraction contains dsRNA-independent along with dsRNA-dependent histone kinase activity so that the histone phosphorylation assay gives abnormally high values for DAI in this fraction.

In order to estimate the degree of purification of DAI between lysate and DE-80 fraction, we measured translational inhibition produced in a standard assay with hemin-supplemented lysate [containing poly(I)·poly(C) at 15 μg/ml to prevent activation of endogenous DAI (1, 2)] by the addition of known amounts of preactivated lysate or DAI DE-80. The results (not shown) indicated that the specific activity of DAI in the DE-80 fraction is about 50 times higher than in the lysate. If so, the overall purification of DAI from the lysate (ternary complex inhibition assay) would be 50 × 5000 or 250,000-fold.

Fig. 5 shows polyacrylamide/NaDodSO$_4$ gel electrophoretic patterns at various purification stages of DAI. The nearly homogeneous enzyme (track 5) migrated slightly behind a 68,000-dalton marker. To see whether the homogeneous active DAI is phosphorylated, DAI DE-80 was activated in the presence of [γ-^{32}P]ATP and purified as described above. Fig. 6 shows that this was indeed the case. The stoichiometry of phosphorylation has not been determined.

Highly purified DAI phosphorylated both the eIF-2 α subunit and histone IIa, as shown in Fig. 7. The figure also shows that prior treatment of the enzyme with MalNEt abolished DAI activity. Previously we have seen (cf. Fig. 1) that MalNEt can also inhibit DAI activation. It may be noted that no phosphorylation of the 68,000-dalton band is seen in Fig. 7. This is presumably because the DAI, which had been activated with nonlabeled ATP, was already phosphorylated.

Because nearly homogeneous DAI can phosphorylate both eIF-2 α and histone (Table 1) it appears that, unlike HCI, DAI can utilize both substrates. This possibility is strengthened by the data of Table 2. They show that histone IIa inhibits phosphorylation of the eIF-2 α subunit by DAI. It is likely that eIF-2

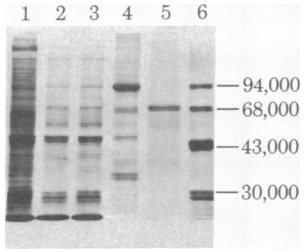

FIG. 5. Purification of preactivated DAI. Polyacrylamide/NaDodSO$_4$ gel electrophoresis of DAI at several steps of purification. Pro-DAI DE-80 was preactivated and purified by chromatography on DEAE-cellulose and affinity chromatography on Sepharose-histone as described in the text. Samples of various fractions were subjected to polyacrylamide/NaDodSO$_4$ gel electrophoresis. Proteins were stained by the silver reduction procedure (27). Track 1, ribosomal salt wash, 130 μg of protein. Track 2, DE-80 before activation, 37 μg of protein. Track 3, DE-80 before activation, 50 μg of protein. Track 4, DE-80, 7.5 μg of protein. Track 5, Sepharose-histone, 4.3 μg of protein. The sample used for electrophoresis was previously concentrated by partial lyophilization after extensive dialysis against distilled water. This may have entailed loss of protein so that the amount actually applied on the gel may have been below the stated 4.3 μg. Track 6, molecular weight markers.

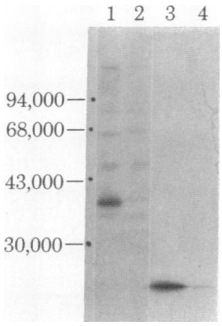

FIG. 7. Phosphorylation of the eIF-2 α subunit and histone IIa by highly purified DAI. Samples were prepared and incubated as described in the text under *Other Assays*. The preactivated DAI (16 ng per sample) was a fraction obtained by DEAE-cellulose chromatography eluted upon raising the concentration of KCl from 0.4 to 1.0 M; its specific activity (ternary complex inhibition assay) at the time of use was 120,000 units/mg. After incubation the samples were subjected to polyacrylamide/NaDodSO$_4$ gel electrophoresis followed by autoradiography. Tracks 1 and 2, eIF-2 as phosphate acceptor without and with prior treatment with MalNEt, respectively. Tracks 3 and 4, histone IIa as phosphate acceptor without and with prior treatment with MalNEt, respectively. Positions of molecular weight markers are shown at the left.

Table 2. Inhibition of eIF-2 α subunit phosphorylation by histone

Control samples			Experimental samples		
Addition	^{32}P bound, cpm	Inhibition, %	Addition	^{32}P bound, cpm	Inhibition, %
None	3500		None	3500	
Albumin, 20 μg	3490	0	Histone, 20 μg	2100	40
Albumin, 40 μg	3530	0	Histone, 30 μg	1480	58
			Histone, 40 μg	610	83

The incubation samples contained 25 mM Hepes buffer at pH 7.6, 4 mM $Mg(OAc)_2$, 0.04 mM [γ-^{32}P]ATP (1000 cpm/pmol), approximately 14 pmol of eIF-2 PC, 2 ng of preactivated DAI (specific activity, 120,000 units/mg), and other additions as indicated, in a final volume of 28 μl. Albumin was from bovine serum. After incubation for 8 min at 30°C, the samples were subjected to polyacrylamide/$NaDodSO_4$ gel electrophoresis as described in the text. The gels were stained with Coomassie blue and the α band of eIF-2 was cut out. The gel section was dissolved in 0.4 ml of H_2O_2 at 95°C and the radioactivity was measured in Hydrofluor. The DAI used here was the same as in Fig. 7.

and histone compete for an active site on the enzyme, although this has not been demonstrated.

DISCUSSION

We have been able to isolate preactivated DAI from reticulocyte lysate as a nearly homogeneous, highly unstable phosphoprotein with an apparent subunit mass of 68,000 daltons. Previously (28, 29) only partial purification of this enzyme had been achieved. The molecular weight of pro-DAI or active DAI was estimated to be about 120,000 (29). Our work supports the view that DAI is a single enzyme that can catalyze the phosphorylation of both histone and the α subunit of eIF-2. This appears to be true also of interferon-induced DAI (11, 12). The fact that virtually homogeneous active DAI is phosphorylated (Fig. 6) confirms earlier suggestions that the enzyme is activated by phosphorylation (1, 2, 28, 29) and is consistent with a model (reactions 1–3)

$$\text{DAI} + \text{dsRNA} \rightarrow \text{active DAI} \quad [1]$$

$$\text{DAI} + \text{ATP} \xrightarrow{\text{(active DAI)}} \text{DAI-}P + \text{ADP} \quad [2]$$

$$\text{eIF-2 (or histone)} + \text{ATP} \xrightarrow{\text{(DAI-}P\text{)}} \text{eIF-2-}P \text{ (or histone-}P\text{)} + \text{ADP} \quad [3]$$

whereby, upon activation by dsRNA, DAI phosphorylates itself and becomes active to phosphorylate its substrates, eIF-2 α and histone. Whether the self-phosphorylation is intramolecular, intermolecular, or both, remains an open question.

Our preliminary results with labeled poly(I)·poly(C) suggest that the first step in DAI activation is dsRNA binding (Fig. 4). This is consistent with the observations that MalNEt blocks both DAI activation (Fig. 1) and dsRNA binding (Fig. 4).

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