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Effect of hemin on site-specific phosphorylation of eukaryotic initiation factor 2

(protein kinase/hemin-controlled repressor/protein synthesis/reticulocyte/Met-tRNA$_f$ binding protein)

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ABSTRACT **Initiation factor 2 (eIF-2) is phosphorylated *in vitro* by two different cyclic nucleotide-independent protein kinases. As previously shown, a protein kinase activity that comigrates with the major casein kinase activity from rabbit reticulocytes phosphorylates eIF-2β. In addition, a second protein kinase that specifically phosphorylates eIF-2α has been identified. Both protein kinase activities demonstrate cyclic nucleotide-independent activity and are not inhibited by the inhibitor protein diagnostic for cyclic AMP-regulated protein kinase activities. Phosphorylation of eIF-2α is almost completely inhibited by 20–35 μM hemin, whereas phosphorylation of eIF-2β is only partially inhibited. Hemin acts by decreasing the rate of incorporation of phosphate into eIF-2α. The protein kinase activity that modifies eIF-2α has been shown to have inhibitory activity in the cell-free protein-synthesizing system, whereas the protein kinase for eIF-2β has no effect. The identity of the former enzyme with the hemin-controlled repressor and role of hemin in the control of initiation are discussed.**

Hemoglobin biosynthesis in both reticulocytes (1) and reticulocyte lysates (2–4) is under the direct control of hemin, which acts as a positive effector for globin synthesis. In the absence of added hemin, protein synthesis is inhibited in a reticulocyte lysate at chain initiation with a concomitant decrease in binding of Met-tRNA$_f$ to 40S ribosomal subunits (5–7). This inhibition is potentiated by addition of ATP (8, 9). Inhibition produced by hemin deprivation is prevented in the reticulocyte lysate by high concentrations of GTP, cyclic AMP, and various purines (8–10). In addition, inhibition of globin synthesis coincides with the appearance of a repressor (11–13). The hemin-controlled repressor (HCR) is thought to be activated from a prorepressor at hemin concentrations suboptimal for translation (5–7, 12, 14–16). Supporting the concept that HCR inhibits initiation is the observation that the addition of initiation factor 2 (eIF-2), the factor that forms a ternary complex with Met-tRNA$_f$ and GTP and then binds to 40S subunits (17–23), will reverse inhibition due to hemin deprivation in the lysate system (24, 25).

eIF-2 is composed of three subunits (25–31) designated α, β, and γ according to a decreasing relative mobility in the gel electrophoresis system of Laemmli (32). Phosphorylation of eIF-2β by a cyclic nucleotide-independent protein kinase has been described (26, 33). It has been reported that purified preparations of HCR possess a protein kinase activity that phosphorylates the small subunit of eIF-2 (29, 34–36). This modification may be correlated with the inhibition of protein synthesis resulting from hemin depletion (34, 37, 38). In these studies we have examined phosphorylation of the two subunits of eIF-2 by the two different protein kinases. The consequences of various low molecular weight effector components, including hemin and cyclic AMP, have been studied on the phosphotransferase reactions.

METHODS AND MATERIALS

[γ-^{32}P]ATP and [γ-^{32}P]GTP were prepared from radioactive orthophosphate (ICN) by a modification of the procedure of Glynn and Chappell (39). Hemin (Schwarz/Mann) was prepared fresh before each experiment and was quantitated as described (13). Homogeneous eIF-2 was prepared as described (40). The heat-stable inhibitor protein was a gift of Donal Walsh, University of California School of Medicine, Davis, CA.

Purification of Protein Kinase Activities. The protein kinase activity for eIF-2β copurified with the major peak of casein kinase activity (casein kinase II; formerly III$_C$) upon chromatography on DEAE-cellulose and phosphocellulose. The initial chromatography on phosphocellulose was done in the absence of monovalent cations in buffer A (25 mM potassium phosphate, pH 7.0/1 mM EDTA/10 mM 2-mercaptoethanol). Under these conditions, the protein kinase activity did not adhere to the resin. When the enzyme was rechromatographed in the presence of 0.25 M NaCl in buffer A, it adhered to the resin and was eluted by 0.75 M NaCl. The protein kinase activity used in these studies to phosphorylate eIF-2α eluted from DEAE-cellulose between 70 and 110 mM KCl and from phosphocellulose between 100 and 200 mM NaCl in buffer A. Details for purification of the protein kinase have been described (41).

Phosphorylation of eIF-2. eIF-2 was phosphorylated in a 0.045-ml reaction volume containing 50 mM Tris·HCl (pH 7.0), 5 mM $MgCl_2$, 0.28 mM [γ-^{32}P]ATP or GTP, and protein kinase. Incubations were initiated by addition of the protein kinase, and mixtures were kept at 30° for 30 min. Phosphorylation was terminated by addition of 0.02 ml of concentrated sample buffer in preparation for analysis by gel electrophoresis.

Slab Gel Electrophoresis. The standard procedure for slab gel electrophoresis (0.75 mm thickness) was used as described, with a modified (26) Laemmli buffer system (32). The concentration of acrylamide in the stacking gel was 3% or 5%, with 7.3% or 10% in the resolving gel as indicated. The acrylamide to bisacrylamide ratio was 37:1 (wt/wt) in both the stacking and resolving gels. The above will be referred to as System I. Electrophoresis was also performed with a different modification of the Laemmli system (42) in which the acrylamide concentration in the stacking and resolving gels was 5% and 15%, respectively, with an acrylamide to bisacrylamide ratio of 38 for

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Abbreviations: eIF-2, eukaryotic initiation factor 2; HCR, hemin-controlled repressor; buffer A, 25 mM potassium phosphate, pH 7.0/1 mM EDTA/10 mM 2-mercaptoethanol.

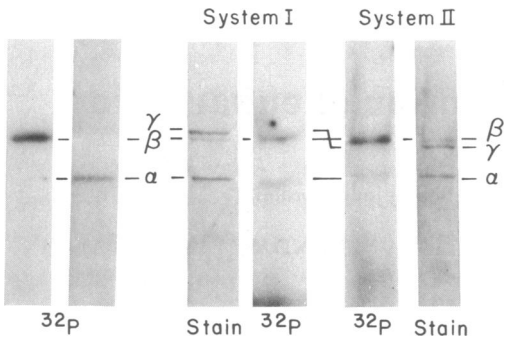

FIG. 1. Comparison of phosphorylated eIF-2 in two denaturing gel electrophoresis systems. Identical samples of eIF-2 (1.8 μg) were phosphorylated with the protein kinase activity specific for eIF-2α (7–15 μg), the protein kinase cochromatographing with casein kinase II (0.5 μg), or with a mixture of two kinases. Gel electrophoresis System I was used in lanes 1–4 (left to right) and System II in lanes 5 and 6. Lane 1, autoradiogram of eIF-2 phosphorylated by casein kinase II; specific activity of ATP, 162 cpm/pmol. Lane 2, autoradiogram of eIF-2 phosphorylated by the enzyme specific for eIF-2α; specific activity of ATP, 676 cpm/pmol. Lanes 3 and 5, stained protein pattern of eIF-2. Lanes 4 and 6, autoradiogram of eIF-2 phosphorylated by a mixture of the protein kinases; specific activity of ATP, 368 and 102 cpm/pmol, respectively.

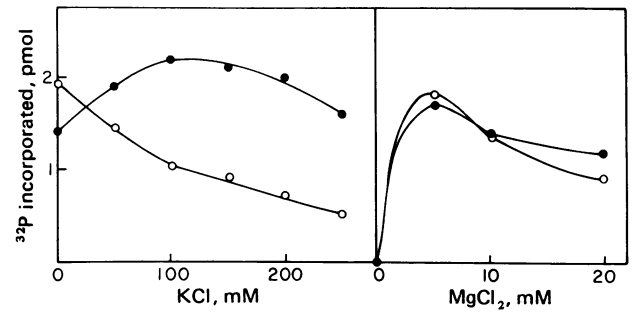

FIG. 2. $MgCl_2$ and KCl requirements for phosphorylation of eIF-2. eIF-2 (1.4 μg) was incubated with [γ-^{32}P]ATP and the appropriate purified protein kinase as described in the legend of Fig. 1. The phosphorylated proteins were analyzed by gel electrophoresis and autoradiography. No salts were added with the initiation factor or the protein kinase. (*Left*) KCl concentration was increased from 0 to 250 mM; $MgCl_2$ was held constant at 10 mM. (*Right*) $MgCl_2$ was varied in concentration from 0 to 20 mM in the absence of KCl. ○, Phosphorylation of the α subunit; ●, phosphorylation of the β subunit. Specific activity of ATP, 1457 cpm/pmol.

the stacking gel and 167 for the resolving gel. This will be referred to as System II. After electrophoresis, the gels were stained in 0.1% Coomassie Brilliant Blue (Schwarz/Mann) in 50% methanol (vol/vol)/7.5% acetic acid (vol/vol) for 30 min and then destained for approximately the same length of time in the stain solvent. Destained gels were then mounted on sheets of Whatman 3 MM filter paper and dried. Radioactivity was visualized with Kodak No-Screen Medical x-ray film. For quantitation, the appropriate stained bands were excised from the gel and placed in 0.5 ml of 1% sodium dodecyl sulfate overnight. Radioactivity was determined in scintillation cocktail containing toluene/Triton X-100 (2:1 vol/vol). Background corrections were made by slicing identical-sized pieces from control lanes.

RESULTS

Phosphorylation of eIF-2. eIF-2 has three nonidentical subunits (25–31). Two of the three subunits were phosphorylated by two different cyclic nucleotide-independent protein kinase activities (Fig. 1). The phosphorylated subunits were identified by polyacrylamide gel electrophoresis and subsequent autoradiography. These two protein kinase activities were isolated by chromatography on DEAE-cellulose and phosphocellulose. The protein kinase that modified eIF-2α eluted in a broad peak between 75 and 250 mM KCl from DEAE-cellulose and at 250 mM NaCl from phosphocellulose. eIF-2β was modified by a protein kinase activity copurifying with the major peak of casein kinase activity (casein kinase II) after chromatography on DEAE-cellulose and phosphocellulose (26). When electrophoresis was performed in System I by a minor modification (26) of the Laemmli system (32), the apparent molecular weights for the individual subunits of eIF-2 were: 38,000 for α, 53,000 for β, and 57,000 for γ. With a mixture of the two protein kinase activities, both the α and β subunit were phosphorylated.

Using a slightly different modification (42) of the Laemmli system (System II), Farrell *et al.* (34) reported that a cyclic nucleotide-independent protein kinase chromatographically similar to the enzyme phosphorylating eIF-2β modified the most slowly migrating band of eIF-2. Thus, we compared the phosphorylation of eIF-2β in the two polyacrylamide gel systems. As shown in Fig. 1, the most slowly migrating subunit of System I (57,000 molecular weight) became the intermediate migrating species in System II (48,000 molecular weight); no changes in migration were observed with the other two subunits. The middle subunit of eIF-2 in our system (System I) is phosphorylated by the same protein kinase activity as the most slowly migrating subunit in System II. Both Systems I and II use the same buffer system; the only difference lies in the acrylamide concentration in the resolving gel (10% in System II compared to 15% in System II), and the acrylamide to bisacrylamide ratio (38 for System I compared 167 for System II). The decreased amount of crosslinking in System II apparently allows for the differential migration behavior of a single subunit of eIF-2.

Optimal Cation Requirements for Phosphorylation of eIF-2. The two partially purified protein kinase activities were used to determine the optimal cation concentrations for phosphorylation of eIF-2. eIF-2 was incubated with either of the protein kinases under conditions of increasing concentrations of $MgCl_2$ or KCl. There was an absolute requirement for magnesium in the phosphotransferase reactions (Fig. 2). The optimum concentration for magnesium was examined in the absence of KCl, and was 5 mM for the phosphorylation of both subunits of eIF-2. The optimum for KCl was examined with 10 mM $MgCl_2$. Increasing concentrations of KCl inhibited phosphorylation of eIF-2α, whereas phosphorylation of eIF-2β was optimal at 100 mM KCl.

Modification of eIF-2 in the Presence of Effectors of Hemoglobin Synthesis. The inhibition of protein synthesis observed in reticulocyte lysates incubated in the absence of hemin or in the presence of oxidized glutathione can be reversed by addition of various effector compounds, including cyclic AMP, GTP, 2-aminopurine, and hemin (8–10). Since a protein kinase activity that modifies eIF-2 α copurifies with the HCR activity (29, 34–36), we examined the effects of these and other compounds on the phosphorylation of both subunits of eIF-2. The compounds tested included cyclic AMP at two concentrations (1 μM and 1 mM), oxidized glutathione (0.75 mM), 2-aminopurine (1 mM), and hemin (30 μM). Phosphate incorporation was determined by excising the subunits of eIF-2 after gel electrophoresis and determining radioactivity by liquid scintillation spectrometry. Excess protein kinase and nucleotide were added to maximize phosphate incorporation. Under these

Table 1. Phosphorylation of eIF-2 in the presence of various effectors of hemoglobin synthesis

Conditions	^{32}P incorporated, pmol			
	eIF-2β		eIF-2α	
	ATP	GTP	ATP	GTP
Complete	1.8	1.9	2.9	1.3
With hemin (0.03 mM)	0.8	0.9	0.1	0.2
With cyclic AMP (1 mM)	1.4		2.0	
With cyclic AMP (0.001 mM)			2.4	
With 2-aminopurine (1 mM)	1.8		2.0	
With glutathione (0.75 mM)	1.4		2.6	
Without eIF-2	0		0.1	
Without protein kinase	0.1			

eIF-2 (1.8 μg) was phosphorylated by the purified protein kinase activity specific for the β subunit (0.5 μg) or for the α subunit (10 μg). Various compounds were added before initiation of the reaction with the protein kinase. Reactions were terminated by addition of concentrated sample buffer. Phosphorylation was quantitated by electrophoresis of the mixtures on polyacrylamide gels followed by excision and determination of radioactivity of the individual protein bands. The incubation mixtures lacking eIF-2 were subjected to identical treatment as the other samples, and the region of the slab gel corresponding to the migration position of the small subunit was excised and radioactivity was determined. Specific activity of ATP was 300 or 500 cpm/pmol; that of GTP, 1100 or 1500 cpm/pmol. The results with the two different protein kinases were obtained in separate experiments.

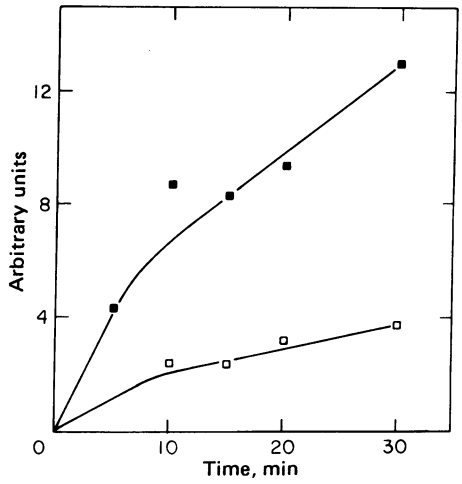

FIG. 3. Effect of hemin on phosphorylation of eIF-2α. eIF-2 was phosphorylated by the protein kinase preparation (15 μg) specific for eIF-2α in the presence (□) and absence (■) of hemin (25 μM). Specific activity for ATP was 143 cpm/pmol. Incubation reactions were terminated at various times with gel sample buffer and analyzed by gel electrophoresis followed by autoradiography. The radioactive phosphate was quantitated by scanning the autoradiogram densitometrically, excising the peak areas, and weighing the paper.

conditions, 1 mol of phosphate was incorporated per mol of eIF-2β. However, in order to examine the role of the various effector compounds on the kinetics of the reaction, we used limiting concentrations of the protein kinase so that less than 15% of the substrate was modified during the reaction. Under these conditions, with 1.8 μg of eIF-2 as substrate, approximately 1 mol of phosphate was incorporated for every 6–7 mol of eIF-2. As shown previously (26, 33), eIF-2β was phosphorylated with either ATP or GTP as the phosphate donor. Both nucleotides are equally effective as phosphate donor molecules in the transfer reaction (Table 1). In the absence of eIF-2 or of protein kinase, no phosphate was incorporated into the 53,000 molecular weight component. The addition of hemin (30 μM) to the reaction depressed the extent of incorporation of phosphate into eIF-2β by about 50%. Cyclic AMP inhibited phosphorylation by approximately 20%, as did oxidized glutathione (0.75 mM). Phosphorylation of eIF-2β in the presence of 2-aminopurine was not affected.

Incorporation of phosphate into eIF-2α was examined with the protein kinase activity specific for this subunit and the same effector compounds described above. Under optimal conditions, up to two phosphoryl moieties can be incorporated into eIF-2α. However, under the kinetic conditions used in the assay, approximately one-fifth of the substrate was modified with ATP in the absence of effector compounds. Table 1 shows that GTP was half as effective as ATP in the phosphotransferase reaction. Cyclic AMP and 2-aminopurine depressed phosphorylation by about 30%, and oxidized glutathione inhibited incorporation by 10%. Concentrations of 30 μM hemin inhibited the phosphorylation reaction greater than 95% with either ATP or GTP. The inhibitory effect produced by hemin on the phosphorylation of eIF-2α was examined further with increasing concentrations of protein kinase or by varying the reaction time. Under conditions of limiting substrate, at concentrations of protein kinase at which eIF-2α was rapidly and maximally phosphorylated, the effect of hemin on the reaction was not as dramatic as that shown in Table 1. This would be expected under conditions under which phosphorylation of eIF-2α is not zero order with respect to the initiation factor. To examine the effect of hemin on the rate of eIF-2α phosphorylation, we conducted the experiment under pseudo-first order conditions so that less than 10% of the initiation factor was phosphorylated during the 30-min incubation period. As shown in Fig. 3, addition of hemin (25 μM) significantly decreased the rate of incorporation of phosphate into eIF-2α. A comparison of initial rates showed that hemin effected a reduction in the phosphorylation of eIF-2α to 25% that of the control. The effect of hemin on eIF-2α phosphorylation appears to be quite specific and cannot be readily attributed to variations in the concentration of free hemin due to nonspecific binding to protein. Since concentrations of hemin required to maintain an optimal rate of protein synthesis in cell lysates range from 20 to 40 μM (2, 3, 11; G. A. Floyd and J. A. Traugh, unpublished results), it is possible that the effect of hemin on protein synthesis can be explained by direct inhibition of the phosphotransferase reaction with eIF-2α. This is supported by experiments examining the effects of hemin on the protein kinase activity in the postribosomal supernatant fraction after formation of HCR. The phosphorylation of eIF-2α was inhibited by greater than 50% upon addition of 25 μM hemin to the supernatant fraction after the protein kinase had been activated by incubation in the absence of hemin for 3 hr (J. A. Traugh and T. S. Lundak, unpublished data).

The absence of any stimulation of phosphorylation by cyclic AMP at the two concentrations tested underscores the cyclic nucleotide independence of both protein kinase activities. This has been further demonstrated by assaying the protein kinase activities with eIF-2 in the presence and absence of the heat-stable inhibitor protein from rabbit skeletal muscle (44). By previously established criteria (45), inhibition of protein kinase activity by the inhibitor protein is a diagnostic test for cyclic AMP-regulated protein kinases. eIF-2 was phosphorylated by a combination of both protein kinases in the absence and presence of the heat-stable inhibitor protein under conditions under which cyclic AMP-regulated kinases were inhibited. As shown in Fig. 4, no inhibition of phosphorylation of either subunit of eIF-2 occurred in the presence of the inhibitor protein (lane 5) when contrasted with phosphorylation in the ab-

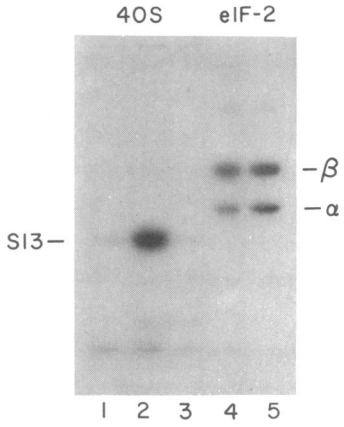

FIG. 4. Effect of heat-stable inhibitor protein on phosphorylation of eIF-2 and 40S ribosomal subunits. Reticulocyte 40S ribosomal subunits (30 μg) and eIF-2 (2.1 μg) were phosphorylated with [γ-^{32}P]ATP (60 Ci/mol) in the presence or absence of heat-stable inhibitor protein. Standard phosphorylation conditions were used with the following exceptions: *N*-morpholinopropylsulfonate (Mops) was used in place of Tris·HCl, 1.4 μM cyclic AMP was included where indicated, and the reaction volume was 0.02 ml. Twenty inhibitor units (44) of the inhibitor protein was added to the incubation where indicated. 40S ribosomal subunits were incubated with 11 enzyme units of cyclic AMP-regulated protein kinase II (43). eIF-2 was incubated with a mixture of cyclic AMP-independent protein kinase activities (1.5 μg). Lanes 1–3 contain 40S subunits and (*1*) no additions; (*2*) cyclic AMP; (*3*) cyclic AMP and inhibitor. Lanes 4 and 5 contain eIF-2 and (*4*) cyclic AMP; (*5*) cyclic AMP and inhibitor.

sence of inhibitor (lane 4). For comparison, 40S ribosomal protein, S13 (38), was phosphorylated by the cyclic AMP-regulated protein kinase in the presence and absence of inhibitor. Essentially no phosphate was transferred in the absence of cyclic AMP (lane 1), and maximal phosphorylation required cyclic AMP (lane 2). As expected, phosphorylation of S13 was inhibited by addition of the inhibitor protein (lane 3). Although both protein kinases were tested in combination, the inhibitor protein had no effect when the individual enzymes were incubated with eIF-2 (data not shown).

Correlation of Protein Kinase Activity with Inhibition of Protein Synthesis. A correlation between incorporation of phosphoryl groups into eIF-2 by the two protein kinases and inhibition of protein synthesis in the cell-free lysate system is shown in Table 2. The protein kinase that phosphorylated eIF-2α inhibited globin synthesis when added to the lysate system. When protein kinase activity was measured by monitoring incorporation of phosphate into the initiation factor, the amount of protein kinase that incorporated 0.45 pmol/15 min into eIF-2α effected a 50% inhibition of protein synthesis. The concentration of protein kinase that incorporated the same amount of phosphate into eIF-2β had no effect on protein synthesis. A 10-fold increase in the amount of protein kinase added to the lysate system was not inhibitory.

DISCUSSION

In this report we demonstrate differences between two cyclic nucleotide-independent protein kinase activities from reticulocytes that are capable of phosphorylating eIF-2. The phosphorylation site for one of these enzymes resides on the α subunit of the initiation factor and is thus distinct from the phosphorylation site for the other protein kinase on the β subunit. Optimal reaction conditions for the phosphorylation of both subunits of eIF-2 have been determined with respect to $MgCl_2$ and KCl concentrations and are distinct for each of the phosphorylation reactions. Neither protein kinase activity is stimulated by cyclic AMP, nor inhibited by the inhibitor protein of Ashby and Walsh (44). Thus, these enzymes are not cyclic AMP-regulated protein kinases or the free catalytic subunit from these enzymes.

Table 2. Correlation of phosphate incorporation into eIF-2 with inhibition of protein synthesis

Enzyme added	^{32}P incorporated, pmol/15 min	% inhibition of protein synthesis
Protein kinase for eIF-2α, 5 μg	0.45	50
Protein kinase for eIF-2β, 0.3 μg	0.13	0
1.0 μg	4.19	0

Increasing amounts of protein kinase were examined for inhibitory activity in the cell-free protein-synthesizing system (13) and for phosphotransferase activity with eIF-2 as substrate (*Methods and Materials*). The degree of inhibition observed with the lysate system was calculated as described (11). The amount of phosphate incorporation into eIF-2 was monitored either by precipitation of the reaction mixture with 10% trichloroacetic acid (43) or by excising the phosphorylated subunits and determining radioactivity in a liquid scintillation counter (*Methods and Materials*). Specific activity of ATP was 200 or 300 cpm/pmol. Concentrations of protein kinase specific for eIF-2α ranged from 0.07 to 7 μg. Concentrations of protein kinase phosphorylating eIF-2β ranged from 0.1 to 2.5 μg. The results with the two different protein kinases were obtained in separate experiments.

Other laboratories have reported phosphorylation of the small subunit of eIF-2 by a protein kinase activity associated with preparations of HCR (29, 34–36). The protein kinase copurifies with HCR through successive stages of purification, and it has been hypothesized that this activity is responsible for the inhibition of translation seen in the absence of hemin or by addition of HCR (34). The HCR-associated protein kinase is cyclic nucleotide independent, although crude preparations of HCR contain a cyclic AMP-regulated protein kinase activity (29, 36). As we have previously shown, purified preparations of cyclic AMP-regulated protein kinases are not capable of phosphorylating any of the three subunits of eIF-2 (26).

It is not immediately apparent if the protein kinase described in this report, which modifies the α subunit of eIF-2, is identical to the protein kinase activity purified as HCR. We have not treated our crude lysates with *N*-ethylmaleimide (29, 34, 46) or heat (35, 36) prior to isolation of the enzymes; such procedures are routinely used prior to isolation of HCR. In addition, this enzyme activity utilizes both ATP and GTP as a phosphate donor in the phosphorylation of eIF-2α, unlike the activity reported by others, which uses only ATP (34). However, we find that an activity that inhibits translation coelutes with the protein kinase.

The protein kinase activity that modifies the small subunit appears to be highly substrate specific. Other suitable substrates for the enzyme have not been identified, although a protein band in the enzyme preparation with a molecular weight of approximately 90,000 is phosphorylated (see Fig. 4). Of interest is the observation that in preparations of purified HCR a protein of molecular weight 96,000 is also radioactively labeled (34). Farrell *et al.* (34) have hypothesized that the phosphorylated, high molecular weight protein may participate in the inhibitory effect attributed to HCR.

The inhibitory effect exhibited by 20–40 μM hemin on the rate of phosphorylation of eIF-2α is well within the range observed to be optimal for maximal stimulation of translation in reticulocyte lysate systems (2, 3, 11). Recently it has been reported that phosphorylation of eIF-2α results in decreased

binding of the Met-tRNA$_f$-GTP-eIF-2 complex to 40S subunits in the presence of other initiation factors (29, 34, 37). This implicates phosphorylation of eIF-2 in the inhibition of initiation, although the phosphorylation of other components may also be involved (38). Since the initiation factor is present in very small concentrations inside the cell (40), increased protein kinase activity in the absence of hemin would rapidly saturate the phosphorylation site(s) of eIF-2α, whereas inhibition of the phosphotransferase reaction by hemin would be sufficient to allow adequate turnover of the phosphate by phosphoprotein phosphatase, preventing accumulation of phosphorylated factor.

It is possible that hemin acts at more than one site. It could be required to perpetuate an inactive form of HCR, as postulated by several laboratories (11, 34, 47), in addition to inhibiting the activity of the protein kinase directly. In addition, a phosphoprotein phosphatase activity has been shown to be altered by changes in hemin levels (48), although phosphorylated eIF-2 has not been shown to be a substrate for this enzyme. Assuming that phosphorylation of the small subunit of eIF-2 is linked with inhibition of protein synthesis observed under conditions of hemin deprivation, inhibition of phosphorylation of eIF-2 by hemin is consistent with the observed role of hemin as a positive effector of globin synthesis.

To the hypothesis that phosphorylation of eIF-2 renders it inactive as an initiation factor, cyclic AMP, GTP, and 2-aminopurine might be expected to be inhibitory towards phosphorylation of eIF-2α based on the observation that these compounds rescue reticulocyte lysates from inhibition by hemin deficiency or from addition of HCR to nondeprived lysates (8–10). Since we have not observed significant inhibition of phosphorylation by these compounds, this would suggest that the rescue effect must occur either at the dephosphorylation step or at a site other than the phosphorylation of the small or middle subunit of eIF-2.

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