PHOSPHORYLATION OF eIF-2β AND EF-1 BY TWO RIBOSOMAL KINASE FRACTIONS

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SUMMARY

Two ribosomal kinase fractions have been partially purified from reticulocyte lysates by DEAE-cellulose and phosphocellulose column chromatography. These fractions autophosphorylated the majority of the bands and phosphorylated EF-1 and eIF-2β. Addition of GSSG (oxidized glutathione) produced a general decrease in phosphorylation. However, phosphorylation of eIF-2β was enhanced in the presence of GSSG.

Key Words: initiation factor-2, elongation factor-1, phosphorylation, casein kinase I, GSSG

INTRODUCTION

Four of the initiation factors, eIF-2, eIF-3, eIF-4B and eIF-4F and two elongation factors, EF-1 and EF-2 required for protein synthesis are phosphorylated ***in vivo*** in eukaryotes (1). Phosphorylation of the alpha subunit of eIF-2 correlates with the inhibition of protein synthesis in a number of different physiologic states such as heme defficiency, viral infection and heat shock (2). eIF-2β has been shown to be phosphorylated with an S6 kinase from liver, the Ca^{2+} - dependent protein kinase and casein kinase II (1).

EF-1 is phosphorylated in A. salina by casein kinase II (3) and by $p34^{cdc2}$ kinase in X. laevis (4). In this study, we report partial purification of two ribosomal salt wash fractions which phosphorylate eIF-β and EF-1. We have determined that these fractions contain casein kinase I activity.

MATERIALS AND METHODS

Materials: Purified preparations of EF-1, CK I and RF were kind gifts of W.C. Merrick, J.A. Traugh and R.L.Matts, respectively. [γ-32p] ATP (3000 Ci/mmol) was obtained from DuPont-New England Nuclear. GSSG, ATP and dithiothreitol were purchased from Sigma.

Partial purification of ribosomal kinase fractions: Reticulocyte lysates supplemented with 10 μM hemin were layered on a 50% glycerol cushion containing Buffer A (10 mM Tris-HCl, pH 7.6, 25 mM KCl, 2 mM Mg $(OAc)_2$ and 5mM NaCl) and centrifuged at 100.000 x g for 20 hours. The pellets were resuspended in Buffer A, brought to 500 mM KCl by the addition of 4 M KCl and centrifuged at 130.000 x g for 2 hours. The supernatant (ribosomal salt wash fraction) was concentrated by ammonium sulfate precipitation at 60% saturation, suspended in Buffer B (25 mM Tris-HCl, pH 7.6, 50 mM KCl, 0.2 mM K-EDTA, 1 mM DTT and 10 % glycerol) and applied to a DEAE cellulose column (2.5 x 20 cm) equilibrated with Buffer B. The fraction eluted by the addition of 0.1 M KCl in Buffer B was concentrated by ammonium sulfate and applied to a phosphocellulose column (1.5 x 18 cm) that was equilibrated with Buffer B. The column was eluted stepwise with Buffer B containing 0.2 M, 0.4 M, 0.6 M and 1.0 M KCl. Fractions eluted with 0.6 M and 1.0 M KCl were termed fraction A and B respectively.

Protein Kinase Assay: Reaction mixtures containing 10 mM HepesKOH, pH 7.6, 3 mM $Mg(OAc)_2$ and 30 μM ATP were pulsed with 5 μCi [γ - ^{32}P] ATP (3000 Ci/mmol) for 10 minutes at 35°C. [^{32}P] phosphoprotein profiles were analyzed as described (5).

RESULTS

Two ribosomal salt wash fractions obtained by chromatography on phosphocellulose (termed A and B) and rich in protein kinase activity autophosphorylated the majority of the bands and phosphorylated EF-1 as well as eIF-2 (Figure 1). Addition of 1 mM oxidized glutathione (GSSG) produced a general decrease in phosphorylation. However, the phosphorylation of eIF-2β by fraction B was enhanced in the presence of GSSG (Figure 2). No eIF-2α kinase activity was present in these fractions. Comparison of the phosphorylation patterns of eIF-2 and RF (eIF-2B) by fraction B and pure casein kinase I indicated that the ribosomal salt wash fraction contained CK-I activity (Figure 3). eIF-2γ was also efficiently phosphorylated by CK-I.

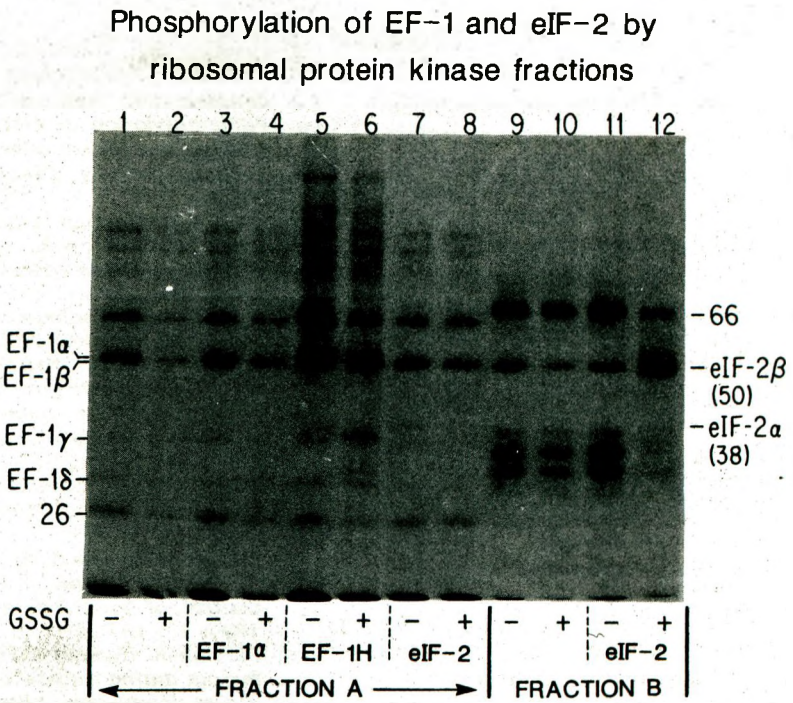

Fig 1. Phosphorylation of EF-1 and eIF-2 by ribosomal protein kinase fractions. *Protein kinase assay was carried out as described under "Materials and Methods". Where indicated, incubation mixtures contained 6 pmol EF-1α and EF-1 H (α, β, γ), 4 pmol eIF-2, 0.5 μg fraction A and B and 2 mM GSSG. Samples not treated with GSSG contained 1 mM dithiothreitol (DTT). Incubation mixtures were each pulsed with 5 μCi [γ-^{32}P] ATP (3000 Ci/mmol) for 10 min. An autoradiogram is shown.*

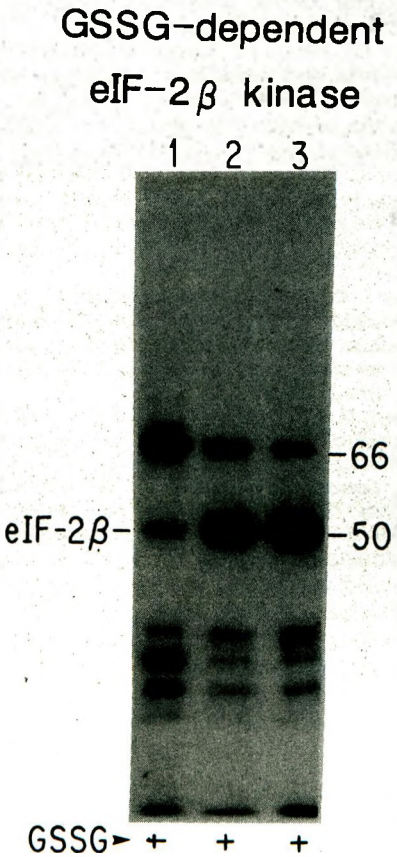

Fig 2. GSSG-dependent eIF-2β kinase. *Protein kinase mixtures containing 0.2 μg fraction B, 2 mM GSSG and increasing amounts of eIF-2 (Lane 1, 2 pmol: Lane 2, 4 pmol: Lane 3, 6 pmol) were incubated as described under Fig 1. and "Materials and Methods". The figure is an autoradiogram.*

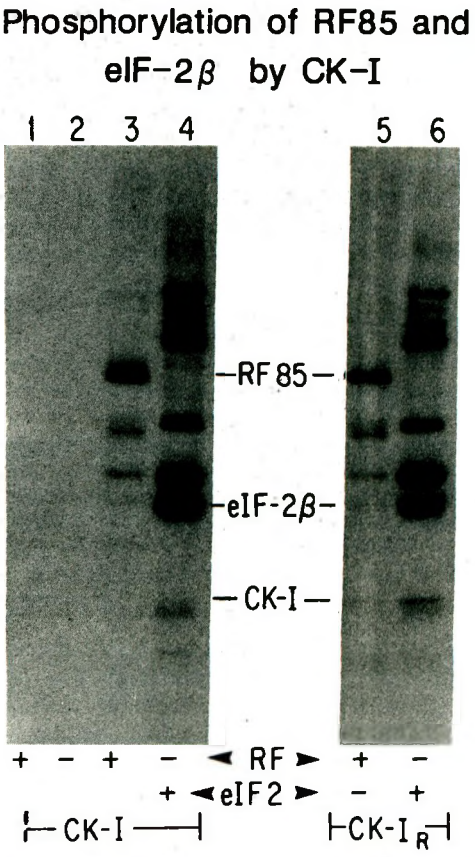

Fig 3. Phosphorylation of RF85 and eIF-2β by CK-I. *Reaction mixtures containing either 0.4 pmol RF (eIF-2B) or 4 pmol eIF-2 were incubated with either 0.01 U pure casein kinase I or 0.2 μg Fraction B (CK-I$_R$) as described under "Materials and Methods". An autoradiogram is shown.*

DISCUSSION

Covalent modification of proteins by phosphorylation comprises one of the modes of regulation of protein synthesis in eukaryotes (6). The inhibiton of protein synthesis in reticulocytes in heme defficiency is associated with phosphorylation of initiation factor 2, eIF-2α (7). Altered translation rates due to phosphorylation of eIF-2α and eIF-4B occur during starvation (8). Upon heat shock (9) and during mitosis (6) eIF-4E (eIF-4F) is dephosphorylated. EF-1 is phosphorylated in Artemia (3) and the recycling activity of EF-1 βγ is enhanced by phosphorylation ***in vivo*** with phorbol ester (10) or ***in vitro*** with protein kinase C (11). EF-2 is specifically phosphorylated by calcium calmodulin dependent protein kinase III (12). Phosphorylation of EF-2 reduces its capacity in translocation (13).

We have partially purified two ribosomal kinase fractions which phosphorylate EF-1 and EF-2β. It has previously been shown that the activity of EF-1 can be regulated by a number of kinases. The β and γ subunits of eIF-2 have been implicated to take part in GTP and met-tRNA$_i$ binding. Phosphorylation of eIF-2β with S6 kinase, Ca^{2+} dependent protein kinase and casein kinase II has previously been shown (1). We have observed a GSSG-dependent eIF-2β kinase activity in fraction B which contains casein kinase I. eIF-2α phosphorylation is one of the best characterized regulatory mechanisms of protein synthesis in eukaryotes. The heme regulated eIF-2α kinase is also activated in GSSG-treated reticulocyte lystates (14). Phosphorylation of eIF-2β under oxidative conditions suggests a second possible regulatory mode involving eIF-2. The physiological effect of EF-1 and eIF-2β phosphorylation by CK-I remains to be determined.

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