

Phospholipid-sensitive Ca^{2+} -dependent protein kinase phosphorylates the β subunit of eukaryotic initiation factor 2 (eIF-2)

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The ability of homogeneous phospholipid-sensitive Ca^{2+} -dependent protein kinase (PL-Ca-PK) from pig spleen to phosphorylate eukaryotic initiation factor 2 (eIF-2) was examined. PL-Ca-PK phosphorylated the β -subunit of eIF-2, whereas myosin light chain kinase (MLCK) and cyclic AMP- and cyclic GMP-dependent protein kinases (cA-PK and cG-PK) did not. PL-Ca-PK could incorporate a maximum of 1.6 mol phosphate/mol eIF-2. The app. K_m and V_{max} for PL-Ca-PK phosphorylation of eIF-2 were $0.13 \mu\text{M}$ and $0.02 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg enzyme}^{-1}$, respectively. Phosphoamino acid analysis revealed that incorporation of phosphate into eIF-2 occurred almost exclusively at serine residues. These findings indicate that eIF-2 was an effective substrate for PL-Ca-PK, suggesting that this enzyme may play a role in the regulation of protein synthesis.

Ca^{2+} Phospholipid Protein synthesis Protein kinase eIF-2 β -Subunit

1. INTRODUCTION

eIF-2 has been identified as one of the factors involved in regulating protein synthesis [1]. This factor forms a ternary complex with Met-tRNA_f and GTP [2] and is composed of 3 non-identical subunits designated α , β and γ of M_r 32000–37000, 35000–59000 and 52000–55000, respectively [3]. Modification of the phosphorylation state of the α -subunit of eIF-2 by 3 separate protein kinases (i.e., hemin-controlled repressor [1], double-stranded RNA-activated inhibitor [1], and protein kinase 380 [4]) has been implicated in the inhibition of protein synthesis. Phosphorylation of eIF-2 α may affect either its ability to bind

Met-tRNA_f and GTP directly [5] or the interaction of this ternary complex with other protein factors also involved in protein synthesis [1]. The β -subunit of eIF-2 has been demonstrated to be phosphorylated by casein kinase II [6] and protease-activated kinase II [5]. The importance of the phosphorylation of the β -subunit is not clear at the present time. In an effort to identify substrates of phospholipid-sensitive Ca^{2+} -dependent protein kinase [7–9] we examined the ability of PL-Ca-PK to phosphorylate eIF-2. Here, we report that PL-Ca-PK phosphorylates the β subunit of eIF-2 with equal effectiveness as the other protein kinases above.

2. EXPERIMENTAL

2.1. Materials

Phosphatidylserine (bovine brain), phosphoserine, phosphothreonine, phosphotyrosine, and histone H1 (type III-S), were purchased from

Abbreviations: eIF-2, eukaryotic initiation factor 2; PL-Ca-PK, phospholipid-sensitive Ca^{2+} -dependent protein kinase; MLCK, myosin light chain kinase; cA-PK and cG-PK, cyclic AMP and cyclic GMP-dependent protein kinases, respectively

Sigma (St Louis MO). Calmodulin was purchased from Sciogen (Detroit MI).

2.2. Methods

eIF-2 was purified to homogeneity from rabbit reticulocytes [3,10] followed by dephosphorylation with alkaline phosphatase [5]. PL-Ca-PK was purified to homogeneity from pig spleen and assayed as in [11]. The standard assay system for PL-Ca-PK, in 0.2 ml, contained 5 μ mol Pipes (pH 6.5), 2 μ mol $MgCl_2$, 5 μ g phosphatidylserine, 0.11 μ g PL-Ca-PK, 1 nmol [γ - ^{32}P]ATP (except in the determination of K_m and V_{max} where 10 nmol [γ - ^{32}P]ATP was used), containing 0.5 – 1.5×10^6 cpm, in the presence or absence of 1 μ mol $CaCl_2$, and with added eIF-2 or histone as protein substrates in the amounts indicated in the individual figure legends. MLCK and cA-PK from bovine heart and cG-PK from bovine lung were purified to homogeneity and their activities assayed [12–14]. SDS–polyacrylamide gel electrophoresis was performed as in [10] using 12.5% acrylamide and a bisacrylamide to acrylamide ratio of 1:118. Phosphoamino acid analysis was performed at pH 1.9 essentially as in [15] in glacial acetic acid/formic acid (88%, v/v) H_2O , 78:25:897 (by vol.).

3. RESULTS AND DISCUSSION

Homogeneous spleen PL-Ca-PK was able to phosphorylate the β -subunit of eIF-2 (fig.1) and to a much lesser extent the γ -subunit. Phosphorylation by PL-Ca-PK occurred only in the combined presence of $CaCl_2$ and phosphatidylserine. In comparison, eIF-2 was not phosphorylated by MLCK, cA-PK, or cG-PK under the conditions employed. It is important to note that all of the studies described herein utilize eIF-2 which was treated with alkaline phosphatase; such treatment has been shown to totally dephosphorylate eIF-2 [5].

The stoichiometry of phosphorylation of eIF-2 by PL-Ca-PK was investigated (fig.2). PL-Ca-PK was able to incorporate a maximum of 1.6 mol phosphate/mol eIF-2. In these studies the enzyme was not a limiting factor since addition of fresh enzyme after 60 min incubation did not result in an increase in phosphate incorporation (fig.2). Casein kinase II and protease-activated kinase II have been reported to incorporate 2 and 1 mol phos-

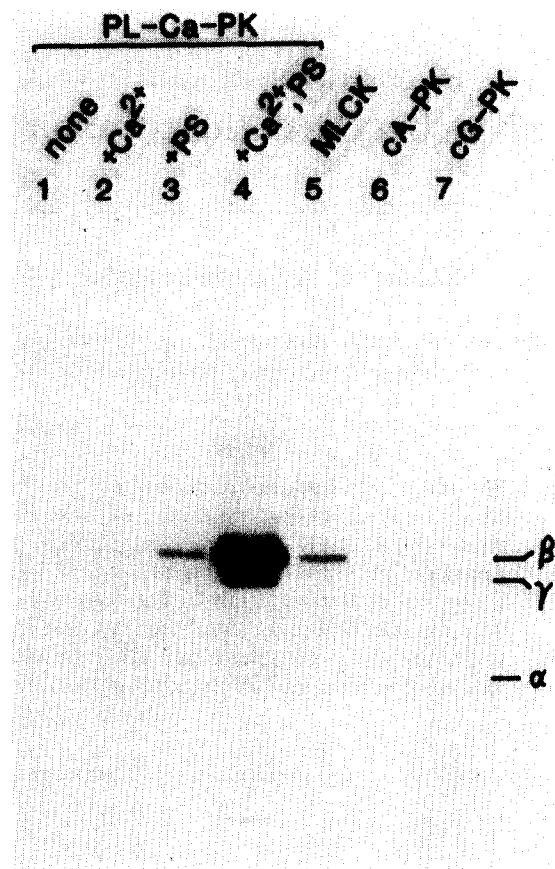


Fig.1. Autoradiograph of phosphorylation of eIF-2 by various protein kinases: eIF-2 (3 μ g) was incubated in 0.1 ml at 30°C for 10 min with PL-Ca-PK (0.11 μ g, lanes 1–4), in the presence or absence of $CaCl_2$ (500 μ M) and/or phosphatidylserine (PS, 5 μ g); MLCK (1.5 μ g) in the presence of $CaCl_2$ (100 μ M) and calmodulin (0.025 μ g); cA-PK (0.3 μ g) or cG-PK (0.5 μ g) in the presence of cyclic AMP or cyclic GMP (both 0.5 μ M), respectively. The separating gel was 12.5% acrylamide containing 0.1% SDS. The amount of eIF-2 applied was 2 μ g/lane.

phate/mol β -subunit of dephosphorylated eIF-2, respectively [5].

The apparent K_m and V_{max} values for eIF-2 of PL-Ca-PK were determined to be 0.13 μ M and 0.02 μ mol $\cdot$ min $^{-1}$ $\cdot$ mg enzyme $^{-1}$, respectively (table 1). This compares with the K_m of 0.30 μ M and V_{max} of 0.06 μ mol $\cdot$ min $^{-1}$ $\cdot$ mg enzyme $^{-1}$ of PL-Ca-PK for histone H1 [11]. The kinetic parameters of the phosphorylation of eIF-2 by PL-Ca-PK and by casein kinase II are also compared

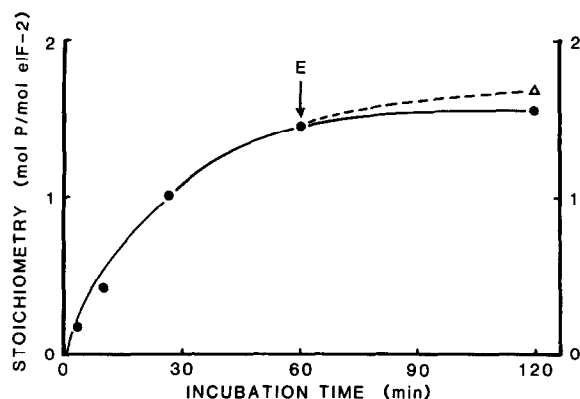


Fig.2. Stoichiometry of phosphorylation of eIF-2 by PL-Ca-PK. eIF-2 (2 μ g) was incubated (0.2 ml) with PL-Ca-PK (0.11 μ g) as in section 2. The data presented were corrected for the basal level seen in the absence of CaCl_2 , which was about 5% of that seen in the presence of CaCl_2 . An eIF-2 M_r of 122000 was used for calculating stoichiometry. The amount of fresh enzyme (E), the same as originally present, was added to certain tubes (...) at 60 min incubation time as indicated by the arrow.

(table 1). It is worth noting that eIF-2 and histone H1 were of comparable effectiveness as substrates for PL-Ca-PK. Histone H1 is the prototype substrate used in the characterization of PL-Ca-PK [11]. Further, both PL-Ca-PK and casein kinase II phosphorylate eIF-2 with comparable effectiveness (table 1).

The nature of the amino acids phosphorylated in eIF-2 by PL-Ca-PK was investigated (fig.3). The results demonstrated that PL-Ca-PK

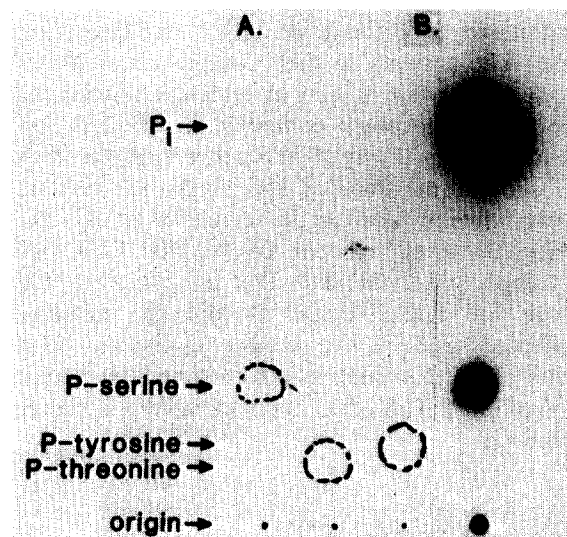


Fig.3. Phosphoamino acid analysis of eIF-2 phosphorylated by PL-Ca-PK. eIF-2 (10 μ g) was phosphorylated by PL-Ca-PK, hydrolyzed and subjected to thin layer chromatography as in section 2: (A) location of standard phosphoamino acids; (B) autoradiogram.

phosphorylated eIF-2 almost exclusively at serine residues. This compares with the ability of PL-Ca-PK to phosphorylate serine and threonine residues in histone H1 [16]. Phosphorylation of eIF-2 by casein kinase II was also reported to occur at serine residues [5].

These studies indicate that PL-Ca-PK could effectively phosphorylate the β -subunit of eIF-2. This suggests that PL-Ca-PK and, therefore, Ca^{2+} and phospholipid, may be involved in the regulation of protein synthesis. The function of phosphorylation of the β -subunit is not yet clear, although phosphorylation of the α -subunit by several protein kinases has been shown to be associated with inhibition of protein synthesis [1,4]. Phosphorylation of the β -subunit may affect the rate of phosphorylation or dephosphorylation of the α -subunit or affect the formation of the ternary initiation complex [$\text{Met-tRNA}_f \cdot \text{GTP} \cdot \text{eIF-2}$] or the subsequent binding of the ternary complex to the 40 S ribosomal subunits [5]. However, the phosphorylation state of the β -subunit did not affect the app. K_m of either the heme-regulated kinase or casein kinase II for eIF-2 in [17]. Whether PL-Ca-PK phosphorylates eIF-2 in vivo,

Table 1

Kinetic parameters of phosphorylation of eIF-2 β and histone H1

Substrate	Protein kinase	K_m (μM)	$V_{\max}$ ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)
eIF-2 β	PL-Ca-PK ^a	0.13	0.020
eIF-2 β	Casein K II ^b	1.1	0.083
Histone H1	PL-Ca-PK ^c	0.30	0.061

^a This study ^b from [17] ^c from [11]

and thereby modifies its activity, is not clear at this time. The answers to such questions may be difficult to ascertain in view of evidence showing that the 2 mol phosphate contained in eIF-2 in vivo turn over very slowly [18]. Further, changes in the phosphorylation state of the β -subunit have not been shown involved in the regulation of eIF-2 activity. However, it is conceivable that PL-Ca-PK could phosphorylate sites that are different from those for casein kinase II and the protease-activated kinase II, thus modifying directly the activity of eIF-2 or indirectly by altering the interaction of eIF-2 with other factors involved in protein synthesis.

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