GDP as a Regulator of Phosphorylation of Elongation Factor 1 by Casein Kinase II†

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Received December 7, 1993; Revised Manuscript Received May 5, 1994®

ABSTRACT: Elongation factor 1 (EF-1) consists of four subunits: the α subunit catalyzes the GTP-dependent binding of aminoacyl-tRNA to ribosomes while the β, γ, and δ subunits catalyze GDP/GTP exchange on EF-1α. Phosphorylation of the β subunit of EF-1 from rabbit reticulocytes by casein kinase II was stimulated up to 22-fold by polylysine, while basic proteins or polyarginine enhanced phosphorylation to a lesser extent. When physiological components of protein synthesis were examined as potential modulators of phosphorylation, ribosomal subunits had no effect, tRNA and poly(U) inhibited the phosphotransferase reaction, and GDP stimulated the initial rate of phosphorylation of EF-1β up to 3.8-fold; the degree of stimulation could be correlated with the amount of α subunit present in EF-1. No stimulation was observed with other nucleotides. Phosphorylation of EF-1β was on serine, and two-dimensional phosphopeptide mapping showed a single tryptic phosphopeptide in the presence of GDP or polylysine; the peptide was identical to that obtained with EF-1 phosphorylated in reticulocytes incubated with [^{32}P]orthophosphate. EF-1δ was also phosphorylated by casein kinase II, but only in the presence of GDP. Kinetic data showed GDP stimulated phosphorylation by increasing the V_{max} with both the β and δ subunits. The GDP-dependent stimulation of phosphorylation was specific for EF-1 and was not observed with calmodulin, β-casein B, or c-Myc. When the catalytic subunit of casein kinase II, cloned and expressed in *Escherichia coli*, was used, only EF-1β was phosphorylated; stimulation by GDP was approximately the same as that observed with the holoenzyme. Since guanine nucleotides bind only to EF-1α, GDP could regulate phosphorylation of EF-1β and δ only when present in the EF-1$\alpha\beta\gamma\delta$ complex. Thus, phosphorylation of EF-1β and δ by casein kinase II would occur primarily at a specific point in the elongation cycle, when EF-1α·GDP is associated with EF-1$\beta\gamma\delta$ following the GTP-dependent binding of aminoacyl-tRNA to the ribosome.

A number of components involved in protein synthesis have been shown to be phosphorylated, including ribosomal proteins, elongation factors (EF),[1] initiation factors (eIF), and aminoacyl-tRNA synthetases (Proud, 1992; Traugh, 1989; Hershey, 1989). Recently, attention has focused on regulation of the elongation phase of protein synthesis catalyzed by EF-1 and -2 (Slobin, 1991; Riis et al., 1990; Nygard & Nilsson, 1990). Purified EF-1 has been isolated from a variety of eukaryotes and consists of three or four subunits. EF-1α mediates the GTP-dependent binding of aminoacyl-tRNA to ribosomes, while the $\beta\gamma$ subunits catalyze exchange of GDP for GTP on EF-1α. Recently, the δ subunit has been shown to have homology with the β subunit and to contain guanine nucleotide exchange activity (Van Damme et al., 1990; Morales et al., 1991). A significant proportion of EF-1 has been isolated in a stable complex with valyl-tRNA synthetase, which elutes with a molecular weight of around 0.8×10^6 upon gel filtration (Motorin et al., 1988; Bec et al., 1989; Bec & Waller, 1989; Venema et al., 1991a).

Although EF-1 is a major constituent of cells, comprising approximately 5% of the total cytosolic protein, only recently has the factor been shown to be phosphorylated *in vivo*. An examination of EF-1 complexed with valyl-tRNA synthetase shows three of the four subunits of EF-1 (α, β, and δ) are phosphorylated in reticulocytes in response to phorbol 12-myristate 13-acetate (Venema et al., 1991a). The same subunits are modified *in vitro* by protein kinase C (Venema et al., 1991b). Phosphorylation *in vivo* and *in vitro* stimulates poly(U)-directed polyphenylalanine synthesis by 2–3-fold (Venema et al., 1991a,b). In the absence of phorbol ester, significant amounts of phosphate are present in the β and δ subunits of EF-1 (Venema et al., 1991a), indicating phosphorylation by another protein kinase(s). In *Xenopus* oocytes, the γ subunit of EF-1 is phosphorylated during M phase, which correlates with the appearance of maturation promoting factor (Mulner-Lorillon et al., 1989). Phosphorylation of EF-1γ is carried out by p34cdc2, a component of maturation promoting factor (Janssen et al., 1991).

Casein kinase II phosphorylates the β subunit of *Artemia salina*, wheat germ, rabbit reticulocytes, and *Xenopus* oocytes (Palen et al., 1990; Janssen et al., 1988; Belle et al., 1989), and the δ subunit has also been reported to be phosphorylated by casein kinase II in oocytes (Belle et al., 1989). EF-1β from *Artemia salina* is phosphorylated on serine-89 by casein kinase II (Janssen et al., 1988), and appears to coincide with a decrease in nucleotide exchange activity. The basic modulatory compound polylysine stimulates phosphorylation of reticulocyte EF-1, but has little effect on phosphorylation of EF-1 from *Artemia* and wheat germ (Palen et al., 1990).

Casein kinase II has been identified in all eukaryotes examined and modifies serine and threonine residues with acidic determinants (glutamic acid, aspartic acid, and/or phosphoamino acid) at the carboxy terminus [for a review, see Pinna (1990) and Tuazon and Traugh (1991)]. The protein kinase activity is stimulated by basic physiological compounds,

† This research was supported by U.S. Public Health Service Grant GM26738.

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® Abstract published in *Advance ACS Abstracts,* June 15, 1994.

[1] Abbreviations: EF, elongation factor; eIF, initiation factor.

0006-2960/94/0433-8515$04.50/0

such as polyamines and basic proteins, and is inhibited by acidic physiological compounds such as heparin and 2,3-bisphosphoglyceric acid. Casein kinase II also appears to be rapidly stimulated in a transient manner in response to growth-promoting compounds and by increased synthesis in the long term (Tuazon & Traugh, 1991).

In this paper, we have examined the effects of potential physiological modulatory compounds on phosphorylation of EF-1 and the EF-1·valyl-tRNA synthetase complex by casein kinase II. The sites of phosphorylation of EF-1 *in vitro* have been compared with the sites modified in reticulocytes labeled with [^{32}P]orthophosphate. GDP has been identified as a modulator, stimulating the initial rate of phosphorylation of EF-1β and δ by casein kinase II.

EXPERIMENTAL PROCEDURES

Materials. [γ-^{32}P]ATP was from Amersham; [^{32}P]orthophosphate was obtained from ICN. Trypsin (diphenylcarbamyl chloride-treated), poly(L-lysine) hydrochloride (MW 36 500), poly(L-arginine) hydrochloride (MW 40 000), protamine sulfate, and histone IIAS were from Sigma. All nucleotides, including GTPγS and GMP-PNP, were purchased from Boehringer Mannheim Biochemicals. Okadaic acid was from Moana BioProducts; calmodulin was obtained from Ocean Biologics. Ribosomal subunits were purified from rabbit reticulocytes by zonal centrifugation as previously described (Traugh & Porter, 1976). tRNA was purified from rabbit reticulocytes and deacylated according to Pendergast and Traugh (1985). Isolation of globin mRNA from rabbit reticulocytes was performed as described previously (Palen & Traugh, 1987). Casein kinase II from rabbit reticulocytes was purified as described previously (Palen & Traugh, 1991). The catalytic subunit (α) of casein kinase II from *Drosophila*, cloned and expressed in *Escherichia coli*, was purified to homogeneity (Lin et al., 1991). Polyclonal antibody prepared against reticulocyte EF-1 in goat was the gift of Dr. William C. Merrick, Case Western Reserve University School of Medicine, Cleveland, OH. β-Casein B was the gift of Elizabeth Bingham, Eastern Regional Research Center, Philadelphia, PA, and c-Myc was generously provided by Dr. Chi Dang, Johns Hopkins University School of Medicine, Baltimore, MD.

Phosphorylation of EF-1$\alpha\beta\gamma\delta$ by Casein Kinase II. EF-1 and EF-1·valyl-tRNA synthetase were purified from rabbit reticulocytes by gel filtration on BioGel A-5m and affinity chromatography on tRNA–Sepharose; further purification to apparent homogeneity by FPLC on Mono Q resulted in a 60–80% depletion of the α subunit (Venema et al., 1991a,b; Venema, 1991). Phosphorylation of EF-1 was measured in 0.070 mL reaction mixtures containing 50 mM Tris-HCl, pH 7.4, 50 mM KCl, 10 mM $MgCl_2$, 0.14 mM [γ-^{32}P]ATP (2000 cpm/pmol), 1 μg of EF-1 (either alone or complexed with valyl-tRNA synthetase), and casein kinase II, as indicated. Incubation was for 30 min at 30 °C, and the reactions were terminated on ice by the addition of 0.005 mL of 100 mM ATP followed by gel electrophoresis sample buffer. Phosphorylated EF-1 was analyzed by gel electrophoresis in sodium dodecyl sulfate on 10% polyacrylamide gels (Hathaway et al., 1979). The gels were stained with Coomassie Brilliant Blue R, destained, and dried. ^{32}P-Labeled bands were visualized by autoradiography with Kodak X-Omat AR film. The quantity of ^{32}P incorporated into the individual protein bands was determined by scanning the autoradiogram at 660 nm with a transmission densitometer and/or by Cerenkov counting of the excised gel bands.

The kinetics of phosphorylation of EF-1 were carried out as described above for 3 min with 0.5–4 μg of EF-1·valyl-tRNA synthetase and 12 units of casein kinase II in the presence or absence of 0.5 mM GDP. The data were analyzed using Lineweaver–Burk plots.

Phosphorylation of EF-1 in Reticulocytes Incubated with [^{32}P]Orthophosphate. Rabbit reticulocytes (16 mL) were labeled for 2.5 h with 0.67 mCi/mL [^{32}P]orthophosphate as described previously (Floyd & Traugh, 1980). Phosphorylation was terminated by the addition of ice-cold saline, and the cells were washed and lysed in the presence of protease inhibitors (10 mM EGTA, 1 mM benzamidine, 0.5 mM phenylmethanesulfonyl fluoride, 4 μg/mL leupeptin, and 4 μg/mL antipain) and phosphatase inhibitors (10 mM EDTA, 50 mM β-glycerophosphate, and 1 μM okadaic acid). EF-1 and EF-1·valyl-tRNA synthetase were isolated from the postribosomal supernatant by gel filtration on BioGel A-5m and affinity chromatography on tRNA–Sepharose in the presence of 50 mM β-glycerophosphate (Venema et al., 1991a; Venema, 1991). Following gel electrophoresis, the identity of EF-1 was confirmed by immunoblotting with polyclonal antibody prepared against reticulocyte EF-1 prepared in goat.

Phosphoamino Acid Analysis and Two-Dimensional Phosphopeptide Mapping of EF-1β. The phosphorylated β subunit of EF-1 was excised from the polyacrylamide gel, extensively digested with trypsin, and lyophilized as described previously (Tuazon et al., 1989). For phosphoamino acid analysis, tryptic digests were hydrolyzed for 2 h at 100–110 °C in 4 N HCl and analyzed by electrophoresis on silica gel plates with phosphoamino acid standards (Tuazon et al., 1989). Phosphorylated amino acids were identified by autoradiography and staining with ninhydrin.

Two-dimensional phosphopeptide mapping was also carried out on the tryptic digests of EF-1β (Tuazon et al., 1989). Phosphopeptides were separated by electrophoresis for 1.5 h at 600 V in pyridine/acetic acid/water (10:0.4:90), pH 6.5; in the second dimension, ascending chromatography was performed in butanol/acetic acid/water (3:1:1).

Protein Determination. Protein concentrations were determined according to the method of Bradford (1976) with bovine γ-globulin as a standard.

RESULTS

Examination of Potential Modulatory Compounds on Phosphorylation of EF-1β by Casein Kinase II. EF-1 purified from rabbit reticulocytes contained α, β, γ, and δ subunits. Modulatory compounds were required for significant phosphorylation of the β subunit by casein kinase II. A low level of phosphorylation of EF-1β was observed with casein kinase II alone. Upon addition of polylysine, phosphorylation of the β subunit was greatly stimulated (Figure 1). Up to 0.45 mol of phosphate could be incorporated per mole of EF-1. Stimulation was also observed with polyarginine and basic proteins, such as mixed histone and protamine, but to a lesser extent. Quantification of the stimulatory effects of these basic polypeptides and proteins on the initial rate of phosphorylation was carried out with EF-1 and EF-1·valyl-tRNA synthetase purified from reticulocytes (Table 1). Polylysine stimulated the rate of phosphorylation of the β subunit 22-fold with EF-1 alone and 16-fold with the complex. Polyarginine stimulated phosphorylation 6–11-fold, while the basic proteins enhanced phosphate incorporation by 5–10-fold.

Since basic proteins are major constituents of ribosomes, it was of interest to determine whether the 40S and 60S ribosomal subunits, or other components of protein synthesis, had any stimulatory effects on phosphorylation of EF-1; neither subunit altered the phosphorylation. Three micrograms of

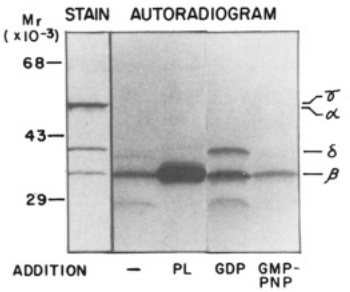

FIGURE 1: Phosphorylation of EF-1 by casein kinase II in the presence of polylysine and guanine nucleotides. Phosphorylation of EF-1 (1 μg) was carried out as described under Experimental Procedures with 55 units of casein kinase II. The reaction mixtures contained 1 μg of polylysine, MW 36 500 (PL), 1 mM GDP, or 1 mM GMP-PNP, as indicated. Phosphorylated EF-1 was analyzed by gel electrophoresis followed by autoradiography.

Table 1: Effects of Basic Polypeptides and Proteins on Stimulation of Phosphorylation of EF-1β by Casein Kinase II[a]

	EF-1		EF-1·ValRS	
effectors	^{32}P incorporated (cpm)	stimulation (x-fold)	^{32}P incorporated (cpm)	stimulation (x-fold)
none	222	1.0	208	1.0
polylysine	4941	22.3	3332	16.0
polyarginine	2412	10.9	1276	6.1
mixed histone (IIAS)	1475	6.6	1097	5.3
protamine	2195	10.1	963	4.6

[a] Phosphorylation reactions were carried out with 1 μg of EF-1 either alone or complexed to valyl-tRNA synthetase (ValRS), 55 units of casein kinase II, and 1 μg of basic polypeptide or protein. The phosphorylated β subunit of EF-1 was analyzed by gel electrophoresis followed by autoradiography, and the radiolabeled subunit was excised from the gel and counted. Phosphate incorporation ranged from 0.34 mol of phosphate/mol of EF-1β (EF-1 with polylysine) to 0.02 mol/mol (no addition).

Table 2: Effects of GDP on Phosphorylation of EF-1β and δ by Casein Kinase II[a]

substrate	subunit	stimulation of phosphorylation (x-fold)
EF-1·ValRS	δ	3.6
	β	3.8
α-depleted EF-1·ValRS	δ	3.1
	β	1.6

[a] EF-1·valyl-tRNA synthetase (ValRS) was purified through tRNA–Sepharose, while α-depleted EF-1·ValRS (0.3 mol of α subunit/mol of EF-1) was purified further by FPLC through Mono Q. Assays contained 60 units of casein kinase II, 100 mM KCl, and 0.5 mM GDP; incubation was for 15 min.

deacylated tRNA or poly(U) inhibited phosphorylation of EF-1β by 40% and 70%, respectively, while globin mRNA had little if any effect. However, addition of GDP resulted in stimulation of phosphorylation of EF-1β either alone (Figure 1), or when added with ribosomal subunits in the presence or absence of globin mRNA (data not shown). Whereas in the presence of basic polypeptides and proteins only the β subunit was modified, in the presence of GDP the δ subunit was also phosphorylated, although to a lesser extent (Figure 1). GMP-PNP, a nonhydrolyzable analogue of GTP, did not affect phosphorylation of the β subunit or promote phosphorylation of the δ subunit of EF-1.

Effects of GDP on Phosphorylation of EF-1. As shown in Table 2, a 3.8-fold stimulation of the β subunit and a 3.6-fold stimulation of the initial rate of phosphorylation of the δ subunit of EF-1 by casein kinase II were observed in the presence of GDP. During the final step of purification, significant

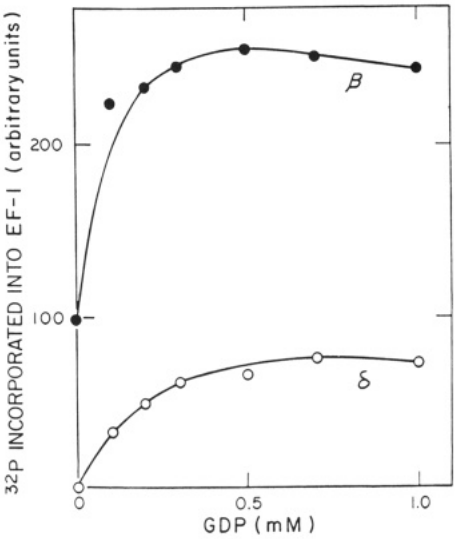

FIGURE 2: Effect of GDP on initial rates of phosphorylation of EF-1. Phosphorylation of EF-1 (1 μg) was carried out with 50 units of casein kinase II under standard conditions, in the presence of increasing concentrations (0–1 mM) of GDP, and analyzed by gel electrophoresis followed by autoradiography. Arbitrary units were calculated from densitometric scans of the autoradiograms. The ratio of the αβγδ subunits of EF-1 was 0.3:1:1:1.

quantities of α subunit were removed (60–80%), resulting in an α-depleted, but highly active form of EF-1·valyl-tRNA synthetase (Venema et al., 1991b). With the α-depleted factor, phosphorylation of the β subunit was stimulated 1.6-fold and of the δ subunit 3.1-fold. Thus, stimulation of the rate of phosphorylation of EF-1β by GDP appeared to be dependent on the amount of α subunit present in the EF-1 complex.

When the initial rate of phosphorylation of the β subunit in highly purified EF-1 was examined at increasing concentrations of GDP, up to a 2.5-fold stimulation was observed, with maximal incorporation obtained around 0.4 mM (Figure 2). Phosphorylation of the δ subunit was approximately 25% that of the β subunit. GDP was required for phosphorylation and was optimal at 0.5 mM GDP. Kinetic analysis of phosphorylation of EF-1 showed that GDP increased the V_{max} for the β subunit from 1055 to 7033 pmol min^{-1} mg^{-1}, but had no effect on the K_m apparent of 1.0 μM. With the δ subunit, the V_{max} was 5274 pmol min^{-1} mg^{-1} in the presence of GDP, with a K_m apparent of 0.6 μM as shown by Lineweaver–Burk analysis. No significant phosphorylation was observed in the absence of GDP.

The specificity of the stimulatory effect of GDP was explored further by examining the effects of a number of nucleoside di- and triphosphates on phosphorylation of EF-1. As shown in Table 3, a 1.8–2.0-fold stimulation of the initial rate of phosphorylation by GDP was observed with EF-1 and EF-1·valyl-tRNA synthetase. ATP, GTP, and GTPγS, at concentrations of 1 mM, inhibited phosphorylation by directly competing with the radiolabeled phosphate donor, [γ-^{32}P]-ATP, present at 0.14 mM; GMP-PNP did not compete with ATP and had no effect on phosphorylation of EF-1 either alone or in the complex. UTP, CTP, ADP, UDP, and CDP had no stimulatory effect, or were inhibitory.

In reticulocytes incubated with [^{32}P]orthophosphate, the α, β, and δ subunits have been shown to be phosphorylated in response to phorbol ester (Venema et al., 1991a). Phosphorylation of the β and δ subunits also occurred in nontreated reticulocytes, but to a lesser extent. Phosphoamino acid analysis of EF-1β from nontreated reticulocytes, or following phosphorylation of purified EF-1 by casein kinase II *in vitro*

Table 3: Effect of Nucleotides on Phosphorylation of EF-1β by Casein Kinase II[a]

	EF-1		EF-1·ValRS	
nucleotide	^{32}P incorporated into EF-1β (cpm)	stimulation (x-fold)	^{32}P incorporated into EF-1β (cpm)	simulation (x-fold)
no addition	679	1.0	654	1.0
polylysine	6567	9.7	6164	9.4
GTP	134	0.2	156	0.2
GDP	1367	2.0	1203	1.8
GMP-PNP	632	0.9	634	1.0
GTPγS	363	0.5	359	0.5
ATP	0	0	0	0
ADP	665	1.0	690	1.1
CTP	779	1.1	669	1.0
CDP	644	0.9	623	1.0
UTP	475	0.7	713	1.1
UDP	366	0.5	577	0.9

[a] Phosphorylation was carried out with 50 units of casein kinase II, 1 μg of EF-1 or EF-1 associated with valyl-tRNA synthetase (ValRS), and 1 μg of polylysine or 1 mM nucleotide, as indicated.

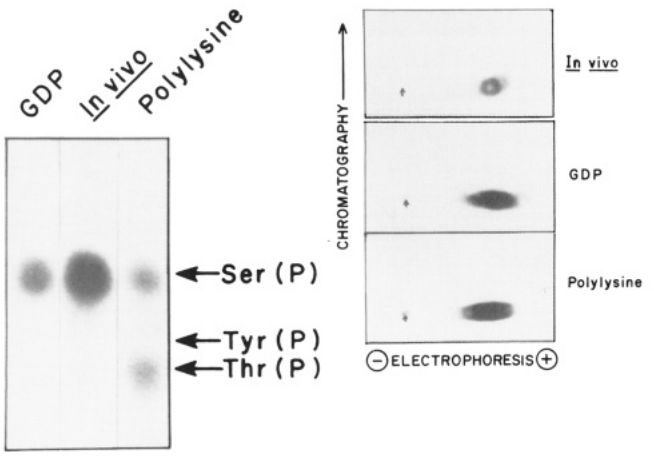

FIGURE 3: Comparison of two-dimensional phosphopeptide maps of EF-1 phosphorylated *in vitro* and *in vivo*. EF-1 was phosphorylated by casein kinase II in the presence of 1 μg of polylysine or 1 mM GDP, or purified from reticulocytes incubated with [^{32}P]orthophosphate (*in vivo*) and analyzed by gel electrophoresis followed by autoradiography as described under Experimental Procedures. The bands corresponding to EF-1β were excised from the gel and subjected to extensive tryptic digestion, and phosphoamino acid analysis (left panel) or two-dimensional phosphopeptide mapping (right panel) was carried out. The autoradiograms are presented. Arrows indicate the origins.

in the presence of GDP, resulted in only phosphoserine (Figure 3, left panel). Upon phosphorylation in the presence of polylysine, the majority of the phosphate was on serine, but a small amount was also observed on threonine. Two-dimensional phosphopeptide mapping of EF-1β phosphorylated in reticulocytes gave a single phosphopeptide. The same phosphopeptide was observed with EF-1 modified *in vitro* in the presence of GDP or polylysine (Figure 3, right panel).

Examination of the Effects of GDP on Phosphorylation of Other Substrates by Casein Kinase II. The effects of GDP on the initial rate of phosphorylation of other substrates were examined and compared with EF-1. Calmodulin (Fukami et al., 1986; Meggio et al., 1987; Nakajo et al., 1988; Lin et al., 1992), β-casein B (Tuazon & Traugh, 1978), and c-Myc (Luscher et al., 1989) have been shown to be phosphorylated by casein kinase II. Phosphorylation of calmodulin by casein kinase II required a basic modulatory compound for phosphorylation to occur (Lin et al., 1992), while phosphorylation of β-casein B and c-Myc was not altered by basic compounds (data not shown). As shown in Table 4, neither GDP nor

Table 4: Effects of Nucleoside Diphosphates on Phosphorylation of Different Substrates by Casein Kinase II[a]

	^{32}P incorporated (x-fold)			
addition	calmodulin	β-casein B	EF-1·ValRS	c-Myc
none	1.0	1.0	1.0	1.0
GDP	0.2	0.8	2.1	0.8
ADP	0.1	0.6	1.1	0.6

[a] Phosphorylation was carried out with 30 units of casein kinase II, 1 mM nucleotide, and 1 μg of calmodulin, β-casein B, EF-1 associated with valyl-tRNA synthetase (ValRS), or 1.5 μg of c-Myc. Following analysis by electrophoresis on 12.5% polyacrylamide gels, the radiolabel was determined by scanning the autoradiograms.

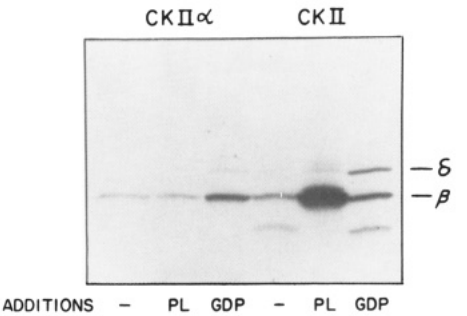

FIGURE 4: Effects of polylysine and GDP on phosphorylation of EF-1 by the catalytic subunit of casein kinase II. Standard assays were carried out with 1 μg of EF-1, 20 units of casein kinase II (CKII) or the catalytic subunit of casein kinase II (CKIIα), and 1 μg of polylysine (PL) or 1 mM GDP, as indicated. The ratio of the $\alpha\beta\gamma\delta$ subunits was 0.2:1:1:1.

ADP had a stimulatory effect on phosphorylation of calmodulin, β-casein B, or c-Myc by casein kinase II, while a 2-fold stimulation of the rate of phosphorylation of EF-1β was observed with GDP.

Phosphorylation of EF-1 by the Catalytic Subunit of Casein Kinase II. Phosphorylation of EF-1 by the catalytic (α) subunit of casein kinase II, cloned, expressed, and highly purified from *E. coli*, was examined and compared with that of the holoenzyme. Using equal amounts of protein kinase activity, as measured with casein, the initial rate of phosphorylation of EF-1β was approximately the same as that observed with the holoenzyme; however, little if any phosphorylation of the δ subunit was observed (Figure 4). Polylysine stimulated phosphorylation of EF-1β by the holoenzyme, but had no effect on phosphorylation by the catalytic subunit, while GDP stimulated phosphorylation to the same extent as that observed with the holoenzyme.

DISCUSSION

Evidence is accumulating that indicates some substrates must be in a correct conformation for phosphorylation to occur and that this conformation is determined by association of substrates with modulatory compounds (Hresko et al., 1990; Thomas et al., 1990; Tuazon et al., 1990). These substrates include phosphoproteins involved in translation. Tuazon et al. (1990) have shown that the cap binding protein, eIF-4E, is significantly phosphorylated by protein kinase C only when present in the cap binding protein complex, eIF-4F. Phosphorylation is at the same site as that modified *in vivo* in response to tumor-promoting phorbol esters (Morley & Traugh, 1989, 1990). Similarly, Palen et al. (1990) have shown that purified EF-1 from reticulocytes is phosphorylated to a significant extent by casein kinase II in the presence of the basic homopolymer polylysine; little phosphorylation is observed in the absence of this compound.

In studies described herein, a search for physiological modulatory compounds which regulate phosphorylation of EF-1 by casein kinase II has been carried out utilizing components involved in protein synthesis. Neither 40S nor 60S ribosomal subunits have any effect on phosphorylation of EF-1, while tRNA and poly(U) significantly inhibit phosphorylation. The latter is to be expected since casein kinase II activity is inhibited by acidic compounds, including RNA (Tuazon & Traugh, 1991). The one physiological compound which stimulates phosphorylation of EF-1β by casein kinase II is GDP; optimal stimulation is observed at 0.4 mM. The rate of stimulation of phosphorylation by GDP correlates with the amount of α subunit associated with purified EF-1. Phosphorylation of the β subunit of EF-1 containing stoichometric amounts of α subunit is stimulated up to 4-fold by GDP, whereas highly purified EF-1, containing only 20–40% of the α subunit, is stimulated 1.5–2.5-fold. This is of interest since the α subunit is the only subunit shown to bind GDP (Caravalho et al., 1984; Anthony et al., 1990). Phosphorylation of EF-1β by casein kinase II *in vitro* is on serine, and the tryptic phosphopeptide is identical to that obtained from reticulocytes incubated with [^{32}P]orthophosphate as shown by phosphopeptide mapping.

Phosphorylation of EF-1β is reduced by addition of nonradioactive ATP, GTP, and GTPγS, all of which compete with [γ-^{32}P]ATP for the protein kinase, since casein kinase II utilizes both ATP and GTP as phosphate donors. Other nucleoside diphosphates and the nonhydrolyzable analogue GMP-PNP have little effect or slightly inhibit phosphorylation (Table 3). Although GMP-PNP binds effectively to EF-1 (Berchtold et al., 1993; Kjeldgaard et al., 1993), it does not compete with [γ-^{32}P]ATP for the catalytic site on casein kinase II. This has been verified with other substrates for the protein kinase (data not shown). Stimulation by GDP appears to be specific for EF-1 and is not observed with three other substrates, calmodulin, β-casein B, or c-Myc.

The δ subunit of EF-1 requires GDP for phosphorylation to occur. It is also phosphorylated in reticulocytes incubated with [^{32}P]orthophosphate (Venema et al., 1991a). Both the β and δ subunits of EF-1 from *Xenopus* oocytes are reported to be modified by casein kinase II *in vitro* (Belle et al., 1989). Sequence analysis of the δ subunit from *Xenopus* shows homology with EF-1β, and EF-1δ contains a recognition sequence for casein kinase II similar to that of the β subunit modified by casein kinase II in *Artemia salina* (Van Damme et al., 1990; Janssen et al., 1988). Since EF-1 from reticulocytes, *Artemia salina*, and wheat germ is modified only on the β and not on the δ subunit in the absence of GDP (Palen et al., 1990), this suggests that the EF-1 preparation from oocytes contained GDP.

When the catalytic subunit of casein kinase II expressed in *E. coli* is utilized, only the β subunit of EF-1 is phosphorylated. GDP stimulates phosphorylation by the catalytic subunit, while no stimulation of phosphorylation is observed with polylysine. The lack of phosphorylation of the δ subunit indicates that the regulatory subunit of casein kinase II is required for recognition of the phosphorylation site in the δ subunit. Similar differential effects on the phosphorylation of other substrates by the regulatory subunit of casein kinase II have been identified previously (Lin et al., 1991, 1992).

The nucleotide binding capacity of EF-1 has been shown to be restricted to the α subunit (Caravalho et al., 1984; Anthony et al., 1990). The GDP form of EF-1α is associated with EF-1$\beta\gamma\delta$, which catalyzes the exchange of GDP bound to EF-1α for GTP, resulting in dissociation of the α subunit from the complex (Slobin & Moller, 1978). The rate of nucleotide exchange catalyzed by EF-1$\beta\gamma\delta$ has been proposed to be a rate-controlling step in eukaryotic translation (Janssen & Moller, 1988; Sherman & Sypherd, 1989). EF-1α·GTP forms a ternary complex with aminoacyl-tRNA and binds aminoacyl-tRNA to the A site on the ribosome to initiate the first step in elongation (Miller & Weissbach, 1977; Slobin, 1991; Riis et al., 1990; Moldave, 1985; Nygard & Nilsson, 1990). Cleavage of GTP results in release of EF-1α·GDP from the ribosome and reassociation with EF-1$\beta\gamma\delta$.

Our data show that phosphorylation of EF-1β and δ by casein kinase II is enhanced by GDP binding to EF-1α, but not by GMP-PNP; hence, EF-1 would be preferentially modified at the stage in the elongation cycle in which EF-1α·GDP is associated with EF-1$\beta\gamma\delta$. It is interesting that the translational initiation factor, eIF-2B, which performs the same role in initiation that EF-1$\beta\gamma\delta$ performs in elongation, may also be regulated by phosphorylation with casein kinase II. eIF-2B is phosphorylated by casein kinase II *in vitro*, resulting in a 5-fold stimulation of nucleotide exchange activity on eIF-2 (Dholakia & Wahba, 1988).

EF-1 is prone to undergo a series of conformational changes during the elongation cycle of protein synthesis as it differentially interacts with GDP, GTP (Berchtold et al., 1993; Kjeldgaard et al., 1993), aminoacyl-tRNA, and ribosomes. Since the conformation of EF-1α is dependent on the type of nucleotide bound, association of EF-1α with GDP appears to specifically expose the recognition sites for casein kinase II on EF-1β and δ, which results in an enhanced rate of phosphorylation by increasing the V_{max}. Similar results were obtained with eIF-4E, showing it is preferentially phosphorylated by protein kinase C when present in the eIF-4F complex (Tuazon et al., 1990). Thus, binding of GDP and not GTP regulates phosphorylation of the two subunits of EF-1 which catalyze GDP/GTP exchange activity, the β and δ subunits. Since EF-1α is always bound to either GDP or GTP, the conformation of EF-1α and the consequent binding to EF-1$\beta\gamma\delta$ are regulated by the type of guanine nucleotide bound. The effects appear to be specific for casein kinase II, and no effect of GDP is observed following phosphorylation of EF-1 by protein kinase C (Y.-W. E. Chang and J. A. Traugh, unpublished results).

ACKNOWLEDGMENT

We thank Dr. William C. Merrick for polyclonal antibody to EF-1, Elizabeth Bingham for β-casein B, Dr. Chi Dang for c-Myc, Theodore T. Huang for excellent technical assistance, William Meek for purification of casein kinase II, and Dr. Wey-Jinq Lin for the α subunit of casein kinase II.

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